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(54) Title: METHODS AND COMPOSITIONS FOR VACCINATION COMPRISING NUCLEIC ACID AND/OR POLYPEPTIDE SEQUENCES OF CHLAMYDIA PSITTACI

(57) Abstract: The instant invention relates to antigens and nucleic acids encoding such antigens obtainable by screening the *Chlamydia psittaci* genome. In more specific aspects, the invention relates to methods of isolating such antigens and nucleic acids and to methods of using such isolated antigens for producing immune responses in bovines or other non-human animals. The ability of an antigen to produce an immune response may be employed in vaccination of bovines or antibody preparation techniques.

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APPLICATION FOR UNITED STATES LETTERS PATENT

for

METHODS AND COMPOSITIONS FOR VACCINATION COMPRISING NUCLEIC ACID AND/OR POLYPEPTIDE SEQUENCES OF *CHLAMYDIA PSITTACI*

by

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BACKGROUND OF THE INVENTION

The government owns rights in the present invention pursuant to DARPA grant number MDA 972-97-1-0013. Incorporated herein by reference is the entire text of the patent application filed on December 17, 2001 in the United States by the same inventors as the present application and entitled "Methods And Compositions For Vaccination Comprising Nucleic Acid And/Or Polypeptide Sequences of *Chlamydia*."

1. Field of the Invention

The present invention relates generally to the fields of immunology, bacteriology and molecular biology. More particularly, the invention relates to methods for screening and obtaining vaccines generated from the administration of expression libraries constructed from a *Chlamydia psittaci* genome. In particular embodiments, it concerns methods and compositions for the vaccination of vertebrate animals against *Chlamydia psittaci* bacterial infections, wherein vaccination of the animal is *via* a protein or gene derived from part or all of the genes validated as vaccines.

2. Description of Related Art

Intracellular bacteria of the genus *Chlamydia* are important pathogens in both man and vertebrate animals, causing blindness in man, sexually transmitted disease, and community-acquired pneumonia, and most likely act as co-factors in atherosclerotic plaque formation in human coronary heart disease.

Ubiquitous *Chlamydia* (*C*) *psittaci* infections in cattle cause mastitis, infertility and abortion. A primary economic impact of *Chlamydia psittaci* in dairy cattle is the loss of milk production and quality. Serological evidence for infection with ruminant *C. psittaci* is found in virtually all cattle (Kaltenbock *et al.*, 1997). These infections typically do not cause overt signs of disease, but under stress of the host animal may elicit transient inflammation of the mammary gland and uterus. These stress-related herd health problems, while not clinically pronounced, result in major losses for animal agriculture due to reduced output and quality of animal products like milk.

Most existing vaccines for the treatment of bacterial infections are composed of live/attenuated or killed pathogens (Babiuk, 1999). Live/attenuated vaccines present the risk of residual, or reacquisition of, pathogenicity, and are associated with a high cost of production. In addition, efficacious live/attenuated vaccines cannot be developed against many pathogens, or are impractical to produce. Killed pathogens typically have less utility than live/attenuated vaccines, as they are not usually effective in eliciting cellular immune responses. An alternative is subunit vaccines that consist of one or a few proteins of the pathogen (Babiuk, 1999; Ellis, 1999). The proteins being developed for these vaccines are typically based on a dominant immune response in infected hosts, and/or on surmised importance in the disease process. Due to the high genetic complexity of bacteria or protozoa, the empirical approach to identify these proteins often requires extensive research on the pathogen's biology and produces a small, biased set of potential vaccine candidates. However, this is currently the only practical method when proteins are the commodity for testing a vaccine.

The development of genetic (DNA) immunization (Tang et al., 1992) not only offers a new method of vaccine delivery, but also enables a new, unbiased, approach to vaccine discovery. One of the inventors proposed that the whole genome of a pathogen could be searched for protein vaccine candidates by directly assessing protection from challenge, termed expression library immunization (ELI) (U.S. Patent 5,703,057, specifically incorporated herein by reference). It involves making an expression library representing the whole genome of the pathogen in a genetic immunization vector. The library is subdivided into smaller groups, and DNA from each library is used to vaccinate animals that are subsequently challenged. The advantage of this approach is that all of the potentially protective genes could be discovered and used in any useful combination to reconstitute a vaccine devoid of non-protective, immunopathological, or immunosuppressive antigens. The potential of ELI was demonstrated in a murine *Mycoplasma pulmonis* infection, against which random *M. pulmonis* libraries were protective (Barry et al., 1995). Since then, others have reported on protective libraries

(Brayton *et al.*, 1998; Piedrafita *et al.*, 1999), but the reduction of these libraries to individual genes has not been demonstrated.

As described above, the widespread human and animal infections by the genus *Chlamydia psittaci* represents a particular challenge for vaccinology. *C. psittaci* infections in cattle cause mastitis, infertility and abortion. A primary economic impact of *Chlamydia psittaci* in dairy cattle is the loss of milk production and quality. Thus, an effective vaccine against *Chlamydia psittaci* bacterial infections in cattle would be of great economic importance. However, there presently have been no effective vaccines developed against *Chlamydia psittaci.*

SUMMARY OF THE INVENTION

In some embodiments, the invention relates to isolated polynucleotides having a region that comprises a sequence of SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:58, or SEQ ID NO:60, a complement of any of these sequences or fragments thereof. In some more specific embodiments, the invention relates to such polynucleotide comprising a region having a sequence comprising at least 17, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 125, 150, 200, or more contiguous nucleotides in common with at least one of SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:58, or SEQ ID NO:60, or its complement. Of course, such polynucleotides may comprise a region having all nucleotides in common with at least one of SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID

NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:58, or SEQ ID NO:60, or its complement.

In other aspect, the invention relates to polypeptides having sequences of SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:59, SEQ ID NO:61 or fragments thereof. The invention also relates to methods of producing such polypeptides using recombinant methods, for example, using the polynucleotides described above.

The invention relates to antibodies against *Chlamydia psittaci* antigens, including those directed against an antigen having sequences of SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, or SEQ ID NO:61, or an antigenic fragment thereof. The antibodies may be polyclonal or monoclonal and produced by methods known in the art.

The invention relates to vaccines for the immunization of an animal against *Chlamydia psittaci*. Such vaccines may comprise a pharmaceutically acceptable carrier, and at least one polynucleotide having a *Chlamydia psittaci* sequence. Such vaccines may be further defined as a vaccine for the immunization of a bovine. Such vaccines may comprise at least one polynucleotide that has a sequence isolated from a *Chlamydia psittaci* genomic DNA expression library. In some preferred embodiments, the vaccine comprises at least one polynucleotide having a sequence of SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID

NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO52:, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO: 58, or SEQ ID NO:60, or fragment thereof. In some specific preferred embodiments, the vaccine comprises at least one polynucleotide having a sequence of SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, or SEQ ID NO:26, or fragment thereof, while in some even more specific embodiments, the vaccine comprises at least one polynucleotide having a sequence of SEQ ID NO:6, SEQ ID NO:10, SEQ ID NO:14, SEQ ID NO:20, or SEQ ID NO:24. The polynucleotide may be comprised in a genetic immunization vector. Some vectors useful in the invention comprise a gene encoding a mouse ubiquitin fusion polypeptide and/or a promoter operable in eukaryotic cells, for example a CMV promoter. The polynucleotide may be cloned into a viral expression vector, for example, a viral expression vector selected from the group consisting of adenovirus, adeno-associated virus, retrovirus and herpes-simplex virus.

In some embodiments, the vaccine comprises at least a first polynucleotide having a *Chlamydia psittaci* sequence and a second polynucleotide having a *Chlamydia psittaci* sequence, wherein the first polynucleotide and the second polynucleotide have different *Chlamydia psittaci* sequences. In some preferred embodiments, the first polynucleotide has a sequence of SEQ ID NO:50.

Other embodiments of vaccines for the immunization of an animal against *Chlamydia psittaci* comprise a pharmaceutically acceptable carrier and at least one *Chlamydia psittaci* antigen; and may be further defined as a vaccine for the immunization of a bovine.. The at least one *Chlamydia psittaci* antigen can be an antigen having a sequence of SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID

NO:55, SEQ ID NO:57, SEQ ID NO:59, or SEQ ID NO:61, or an antigenic fragment thereof. In some specific embodiments, the vaccine comprises at least one *Chlamydia psittaci* antigen having a sequence of SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, or SEQ ID NO:27 or an antigenic fragment thereof. In some even more specific embodiments, the at least one *Chlamydia psittaci* antigen has a sequence of SEQ ID NO:7, SEQ ID NO:11, SEQ ID NO:15, SEQ ID NO:21, or SEQ ID NO:25.

The invention contemplates methods of immunizing an animal comprising providing to the bovine at least one *Chlamydia psittaci* antigen, or antigenic fragment thereof, in an amount effective to induce an immune response. The antigens described above are examples of particularly useful antigens in this regard. The provision of at least one *Chlamydia psittaci* antigen may comprise: (a) preparing a cloned expression library from fragmented genomic DNA, cDNA or sequenced genes of *Chlamydia psittaci*; (b) administering at least one clone of the library in a pharmaceutically acceptable carrier into the bovine; and (c) expressing at least one *Chlamydia psittaci* antigen in the bovine. The polynucleotide may be administered, for example, by a intramuscular injection or epidermal injection or intravenous, subcutaneous, intralesional, intraperitoneal, oral or inhaled routes of administration. An intramuscular injection may comprise least 1.0 μg to 200 μg of the polynucleotide, whereas an epidermal injection may comprise at least 0.01 μg to 5.0 μg of the polynucleotide. Second intramuscular injection or epidermal injections may be administered, for example, at least about three weeks after the first injection. The polynucleotide may be comprised in a viral expression vector.

Alternatively, the provision of the *Chlamydia* antigen(s) may comprise: (a) preparing a pharmaceutical composition comprising at least one polynucleotide having a sequence of SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID

NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO52:, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO: 58, or SEQ ID NO:60, or fragment thereof; (b) administering one or more clones of the library in a pharmaceutically acceptable carrier into the bovine; and (c) expressing one or more *Chlamydia* antigens in the bovine. The antigen may be administered in much the same manner as described for polynucleotides above, and other manners known to those of skill in the art.

In another alternative, the provision of the *Chlamydia* antigen(s) may comprise: (a) preparing a pharmaceutical composition of at least one Chlamydia antigen having a sequence of SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, or SEQ ID NO:61, or an antigenic fragment thereof; and (b) administering the at least one antigen or fragment into the animal.

This specification discloses methods of obtaining polynucleotide sequences effective for generating an immune response against *Chlamydia psittaci* comprising: (a) preparing a cloned expression library from fragmented genomic DNA of the species *Chlamydia psittaci*; (b) administering one or more clones of the library in a pharmaceutically acceptable carrier into the animal in an amount effective to induce an immune response; and (c) selecting from the library the polynucleotide sequences that induce an immune response, wherein the immune response in the animal is protective against *Chlamydia psittaci* infection. Such methods may further comprise testing the animal for immune resistance against a *Chlamydia psittaci* bacterial infection by challenging the animal with *Chlamydia psittaci*. The genomic DNA is fragmented physically or by restriction enzymes, and in some preferred embodiments, the fragments are about 200-1000 base pairs. In some cases each clone in the library may comprise a gene encoding a mouse ubiquitin fusion polypeptide designed to link the expression library polynucleotides to the ubiquitin gene. In some embodiments, the library is about

$1x10^3$ to about $1x10^6$ clones, with some preferred embodiments using a library having $1x10^5$ clones. In some cases, about 0.01 μg to about 200 μg of DNA, cDNA or sequenced gene from the clones is administered into the animal, for example by intramuscular injection or epidermal injection. In many cases, the cloned expression library further comprises a promoter operably linked to the DNA that permits expression in a vertebrate animal cell.

The invention also relates to methods of assaying for the presence of *Chlamydia psittaci* infection in an animal comprising: (a) obtaining an antibody directed against a *Chlamydia psittaci* antigen; (b) obtaining a sample from the bovine; (c) admixing the antibody with the sample; and (d) assaying the sample for antigen-antibody binding, wherein the antigen-antibody binding indicates *Chlamydia psittaci* infection in the bovine. In some embodiments the animal is a bovine. In many cases, the antibody directed against the antigen is a monoclonal antibody. In some preferred embodiments, assaying the sample for antigen-antibody binding is done by precipitin reaction, radioimmunoassay, ELISA, Western blot or immunofluorescence. The invention also relates to kits for assaying animals such as bovines for a *Chlamydia psittaci* infection comprising, in a suitable container: (a) a pharmaceutically acceptable carrier; and (b) an antibody directed against a *Chlamydia psittaci* antigen. The method also relates to a method of assaying for the presence of a *Chlamydia psittaci* infection in a bovine comprising: (a) obtaining an oligonucleotide probe comprising a sequence comprised within one of SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO52:, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO: 58, or SEQ ID NO:60, or a complement thereof; and (b) employing the probe in a PCR detection protocol. Kits for such protocols are also within the scope of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

FIG. 1. Scheme for Expression Library Immunization.

FIG. 2. Production of the *C. psittaci* Library. The *C. psittaci* library was produced by first physically shearing the genomic DNA, strain BGM/B577, and size selecting fragments of 300-800 base pairs. The fragments were ligated into the *Bgl* II site of pCMVi-Ubs(+P3); see Sykes and Johnston, 1999 for details. The nucleotide sequence shown in this figure is given as SEQ ID NO:1.

FIG. 3. Flowchart depicting the process for deconvolution of the libraries. Each round consists of preparation of DNA samples, vaccination of mice, challenge and determination of the relative protection in each group.

FIG. 4. Results of protection assays in Rounds 1, 2 and 3. Protection was scored as lung weight relative to average of the vaccinated, maximum protection, positive control and the non-vaccinated, challenged, maximum disease, negative control. The relative protection score was calculated by assigning the score 1 to animals with lung weight equal to the vaccinated control and the score 0 to animals with lung weights equal to the challenged, non-vaccinated control. These points define a line; animals with lower lung weight, hence better protection, have a higher relative protection score. Animals that have worse disease than challenged, non-vaccinated controls, i.e. heavier lungs, will have a negative relative protection score. The unchallenged Naïve group consistently had lung weights slightly lower than the maximum protection, positive controls (Vaccinated) due to the peribronchiolar accumulation of lymphatic cells. In Rounds 2 and 3 the pools of plasmids from columns in the two-dimensional arrays are assigned numbers and the

rows assigned letters. The solid bars indicate pools that were designated as protective and entered into the subsequent round. The error bars represent one standard deviation on either side of the mean.

FIG. 5. Results of protection assays of testing individual gene fragments in Round 4. Protection was scored as lung weight relative to the average of the vaccinated, maximum protection, positive control (Vaccinated=1) and the non-vaccinated, challenged, maximum disease, negative control (Challenged=0). The Pool<50AA is the DNA consisting of the pool of the 32 plasmids from Round 3 having predicted open-reading frames less than 50 amino acids long. Pool>50AA is the DNA consisting of all the 14 plasmids containing *C. psittaci* inserts encoding in-frame proteins more than 50 amino acids long. The numbers of each individual gene fragment tested correspond to the numbers in FIG. 4. The error bars represent one standard deviation of the mean.

FIG. 6. Summary of characterization of the single gene fragments of Round 4. The Relative Protection score of each *C. psittaci* (CP) gene fragment is provided along with the designation of the gene in *C. pneumonia* that has the highest similarity (*C. pneumonia* homolog). In two cases, gene fragment CP #4 and CP #12, the *C. psittaci* gene could also be identified. On the right is a linear map showing the location in each gene of the fragment that conferred protection (shaded).

FIG. 7. Protection data from DNA pools. CP1-6 is a negative pool from round 1. To test whether a single protective gene could be detected in a negative pool, 25 ng of either CP4 #4 or CP4 #11 was added to 50 μg of CP1-6.

DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

The present invention provides compositions and methods for the immunization of vertebrate animals, including humans, against infections using nucleic acid sequences and polypeptides elucidated by screening *Chlamydia psittaci*. These compositions and methods will be useful for immunization against *Chlamydia psittaci* bacterial infections

and other infections and disease states.. In particular embodiments, a vaccine composition directed against *Chlamydia psittaci* infections is provided. The vaccine according to the present invention comprises *Chlamydia psittaci* genes and polynucleotides identified by the inventors, that confer protective resistance in vertebrate animals to *Chlamydia psittaci* bacterial infections, and other infections. In other embodiments, the invention provides methods for immunizing an animal against *Chlamydia psittaci* infections, methods for preparing a cloned library *via* expression library immunization and methods for screening and identifying *Chlamydia psittaci* genes that confer protection against infection.

A. Expression Library Immunization

In particular embodiments, the immunization of vertebrate animals according to the present invention includes a cloned library of *Chlamydia psittaci* expression constructs. In specific embodiments, a cloned expression library of *Chlamydia psittaci* is provided. Expression library immunization, ELI herein, is well known in the art (U. S. Patent 5,703,057, specifically incorporated herein by reference). In certain embodiments, the invention provides an ELI method applicable to virtually any pathogen and requires no knowledge of the biological properties of the pathogen. The method operates on the assumption, generally accepted by those skilled in the art, that all the potential antigenic determinants of any pathogen are encoded in its genome. The inventors have previously devised methods of identifying vaccines using a genomic expression library representing all of the antigenic determinants of a pathogen (U. S. Patent 5,703,057). The method uses to its advantage the simplicity of genetic immunization to sort through a genome for immunological reagents in an unbiased, systematic fashion.

The preparation of an expression library is performed using the techniques and methods familiar one of skill in the art. The pathogen's genome, may or may not be known or possibly may even have been cloned. Thus one obtains DNA (or cDNA), representing substantially the entire genome of the pathogen (*e.g., Chlamydia psittaci).* The DNA is broken up, by physical fragmentation or restriction endonuclease, into segments of some length so as to provide a library of about 10^5 (approximately 18x the

genome size) members. The library is then tested by inoculating a subject with purified DNA of the library or sub-library and the subject challenged with a pathogen, wherein immune protection of the subject from pathogen challenge indicates a clone that confers a protective immune response against infection.

B. Nucleic Acids

The present invention provides *Chlamydia psittaci* polynucleotide compositions and methods that induce a protective immune response in vertebrate animals challenged with a *Chlamydia psittaci* bacterial infection. The preparation and purification of antigenic *Chlamydia psittaci* polypeptides, or fragments thereof (Section C) and antibody preparations directed against *Chlamydia psittaci* antigens, or fragments thereof (Section E) are described below.

Thus, in certain embodiments of the present invention, genes or polynucleotides encoding *Chlamydia psittaci* polypeptides or fragments thereof are provided. It is contemplated in other embodiments, that a polynucleotide encoding a *Chlamydia psittaci* polypeptide or polypeptide fragment will be expressed in prokaryotic or eukaryotic cells and the polypeptides purified for use as anti-*Chlamydia psittaci* antigens in the vaccination of vertebrate animals or in generating antibodies immunoreactive with *Chlamydia psittaci* polypeptides (*i.e.*, antigens). The genomes of *C. pneumoniae* and *C. trachomatis* have been completely sequenced. The *Chlamydia* genes are quite similar, with the four most protective genes identified being 30-71% identical and 45-85% similar in amino acid sequence.

1. Nucleic Acids Encoding *Chlamydia psittaci* Polypeptides

The present invention provides polynucleotides encoding antigenic *Chlamydia psittaci* polypeptides capable of inducing a protective immune response in vertebrate animals and for use as an antigen to generate anti-*Chlamydia psittaci* or other pathogen antibodies. In certain instances, it may be desirable to express *Chlamydia psittaci* polynucleotides encoding a particular antigenic *Chlamydia psittaci* polypeptide domain or sequence to be used as a vaccine or in generating anti-*Chlamydia psittaci* or other pathogen antibodies. Nucleic acids according to the present invention may encode an entire

Chlamydia psittaci gene, or any other fragment of the *Chlamydia psittaci* sequences set forth herein. The nucleic acid may be derived from genomic DNA, *i.e.*, cloned directly from the genome of a particular organism. In other embodiments, however, the nucleic acid may comprise complementary DNA (cDNA). A protein may be derived from the designated sequences for use in a vaccine or to isolate useful antibodies.

The term "cDNA" is intended to refer to DNA prepared using messenger RNA (mRNA) as template. The advantage of using a cDNA, as opposed to genomic DNA or DNA polymerized from a genomic, non- or partially-processed RNA template, is that the cDNA primarily contains coding sequences of the corresponding protein. There may be times when the full or partial genomic sequence is preferred, such as where the non-coding regions are required for optimal expression.

It also is contemplated that a given *Chlamydia psittaci* polynucleotide may be represented by natural variants that have slightly different nucleic acid sequences but, nonetheless, encode the same polypeptide (see Table 2 below). In addition, it is contemplated that a given *Chlamydia psittaci* polypeptide may be generated using alternate codons that result in a different nucleic acid sequence but encodes the same polypeptide.

As used in this application, the term "a nucleic acid encoding a *Chlamydia psittaci* polynucleotide" refers to a nucleic acid molecule that has been isolated free of total cellular nucleic acid. The term "functionally equivalent codon" is used herein to refer to codons that encode the same amino acid, such as the six codons for arginine or serine (Table 2, below), and also refers to codons that encode biologically equivalent amino acids, as discussed in the following pages.

TABLE 1

Amino Acids			Codons
Alanine	Ala	A	GCA GCC GCG GCU
Cysteine	Cys	C	UGC UGU
Aspartic acid	Asp	D	GAC GAU
Glutamic acid	Glu	E	GAA GAG
Phenylalanine	Phe	F	UUC UUU
Glycine	Gly	G	GGA GGC GGG GGU
Histidine	His	H	CAC CAU
Isoleucine	Ile	I	AUA AUC AUU
Lysine	Lys	K	AAA AAG
Leucine	Leu	L	UUA UUG CUA CUC CUG CUU
Methionine	Met	M	AUG
Asparagine	Asn	N	AAC AAU
Proline	Pro	P	CCA CCC CCG CCU
Glutamine	Gln	Q	CAA CAG
Arginine	Arg	R	AGA AGG CGA CGC CGG CGU
Serine	Ser	S	AGC AGU UCA UCC UCG UCU
Threonine	Thr	T	ACA ACC ACG ACU
Valine	Val	V	GUA GUC GUG GUU
Tryptophan	Trp	W	UGG
Tyrosine	Tyr	Y	UAC UAU

Allowing for the degeneracy of the genetic code, sequences that have at least about 50%, usually at least about 60%, more usually about 70%, most usually about 80%, preferably at least about 90% and most preferably about 95% of nucleotides that are identical to the nucleotides of given *Chlamydia psittaci* gene or polynucleotide. Sequences that are essentially the same as those set forth in a *Chlamydia psittaci* gene or polynucleotide may also be functionally defined as sequences that are capable of

hybridizing to a nucleic acid segment containing the complement of a *Chlamydia psittaci* polynucleotide under standard conditions.

The DNA segments of the present invention include those encoding functional and/or immunologically equivalent *Chlamydia psittaci* proteins and peptides, as described above. Such sequences may arise as a consequence of codon redundancy and amino acid functional equivalency that are known to occur naturally within nucleic acid sequences and the proteins thus encoded. Alternatively, functionally and/or immunogenically equivalent proteins or peptides may be created via the application of recombinant DNA technology, in which changes in the protein structure may be engineered, based on considerations of the properties of the amino acids being exchanged. Changes designed by man may be introduced through the application of site-directed mutagenesis techniques or may be introduced randomly and screened later for the desired function, as described below.

2. Oligonucleotide Sequences

Naturally, the present invention also encompasses DNA segments that are complementary, or essentially complementary to the sequences of a *Chlamydia psittaci* polynucleotide. Nucleic acid sequences that are "complementary" are those that are capable of base-pairing according to the standard Watson-Crick complementary rules. As used herein, the term "complementary sequences" means nucleic acid sequences that are substantially complementary, as may be assessed by the same nucleotide comparison set forth above, or as defined as being capable of hybridizing to the nucleic acid segment of a *Chlamydia psittaci* polynucleotide under relatively stringent conditions such as those described herein. Such sequences may encode the entire *Chlamydia psittaci* polypeptide or functional or non-functional fragments thereof.

Alternatively, the hybridizing segments may be shorter oligonucleotides. Sequences of 17 bases long should occur only once in the human genome and, therefore, suffice to specify a unique target sequence. Although shorter oligomers are easier to make and increase *in vivo* accessibility, numerous other factors are involved in determining the specificity of hybridization. Both binding affinity and sequence specificity of an

oligonucleotide to its complementary target increases with increasing length. It is contemplated that exemplary oligonucleotides of 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100 or more base pairs will be used, although others are contemplated. Longer polynucleotides encoding 250, 500, 1000, 1212, 1500, 2000, 2500, 3000 or 3500 bases and longer are contemplated as well. Such oligonucleotides will find use, for example, as probes in Southern and Northern blots and as primers in amplification reactions, or for vaccines..

Suitable hybridization conditions will be well known to those of skill in the art. In certain applications, for example, substitution of amino acids by site-directed mutagenesis, it is appreciated that lower stringency conditions are required. Under these conditions, hybridization may occur even though the sequences of probe and target strand are not perfectly complementary, but are mismatched at one or more positions. Conditions may be rendered less stringent by increasing salt concentration and decreasing temperature. For example, a medium stringency condition could be provided by about 0.1 to 0.25 M NaCl at temperatures of about 37°C to about 55°C, while a low stringency condition could be provided by about 0.15 M to about 0.9 M salt, at temperatures ranging from about 20°C to about 55°C. Thus, hybridization conditions can be readily manipulated, and thus will generally be a method of choice depending on the desired results.

In other embodiments, hybridization may be achieved under conditions of, for example, 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM $MgCl_2$, 10 mM dithiothreitol, at temperatures between approximately 20°C to about 37°C. Other hybridization conditions utilized could include approximately 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, at temperatures ranging from approximately 40°C to about 72°C. Formamide and SDS also may be used to alter the hybridization conditions.

One method of using probes and primers of the present invention is in the search for genes related to *Chlamydia psittaci* or, more particularly, homologs of *Chlamydia psittaci* from other species. Normally, the target DNA will be a genomic or cDNA library, although screening may involve analysis of RNA molecules. By varying the stringency of

hybridization, and the region of the probe, different degrees of homology may be discovered.

Another way of exploiting probes and primers of the present invention is in site-directed, or site-specific mutagenesis. Site-specific mutagenesis is a technique useful in the preparation of individual peptides, or biologically functional equivalent proteins or peptides, through specific mutagenesis of the underlying DNA. The technique further provides a ready ability to prepare and test sequence variants, incorporating one or more of the foregoing considerations, by introducing one or more nucleotide sequence changes into the DNA. Site-specific mutagenesis allows the production of mutants through the use of specific oligonucleotide sequences which encode the DNA sequence of the desired mutation, as well as a sufficient number of adjacent nucleotides, to provide a primer sequence of sufficient size and sequence complexity to form a stable duplex on both sides of the deletion junction being traversed. Typically, a primer of about 17 to 25 nucleotides in length is preferred, with about 5 to 10 residues on both sides of the junction of the sequence being altered.

The technique typically employs a bacteriophage vector that exists in both a single stranded and double stranded form. Typical vectors useful in site-directed mutagenesis include vectors such as the M13 phage. These phage vectors are commercially available and their use is generally well known to those skilled in the art. Double stranded plasmids are also routinely employed in site directed mutagenesis, which eliminates the step of transferring the gene of interest from a phage to a plasmid.

In general, site-directed mutagenesis is performed by first obtaining a single-stranded vector, or melting of two strands of a double stranded vector which includes within its sequence a DNA sequence encoding the desired protein. An oligonucleotide primer bearing the desired mutated sequence is synthetically prepared. This primer is then annealed with the single-stranded DNA preparation, taking into account the degree of mismatch when selecting hybridization conditions, and subjected to DNA polymerizing enzymes such as *E. coli* polymerase I Klenow fragment, in order to

complete the synthesis of the mutation-bearing strand. Thus, a heteroduplex is formed wherein one strand encodes the original non-mutated sequence and the second strand bears the desired mutation. This heteroduplex vector is then used to transform appropriate cells, such as *E. coli* cells, and clones are selected that include recombinant vectors bearing the mutated sequence arrangement.

The preparation of sequence variants of the selected gene using site-directed mutagenesis is provided as a means of producing potentially useful species and is not meant to be limiting, as there are other ways in which sequence variants of genes may be obtained. For example, recombinant vectors encoding the desired gene may be treated with mutagenic agents, such as hydroxylamine, to obtain sequence variants.

C. Polypeptides and Antigens

For the purposes of the present invention a *Chlamydia psittaci* polypeptide used as an antigen may be a naturally-occurring *Chlamydia psittaci* polypeptide that has been extracted using protein extraction techniques well known to those of skill in the art. In particular embodiments, a *Chlamydia psittaci* antigen is identified by ELI and prepared in a pharmaceutically acceptable carrier for the vaccination of an animal against *Chlamydia psittaci* infection.

In alternative embodiments, the *Chlamydia psittaci* polypeptide or antigen may be a synthetic peptide. In still other embodiments, the peptide may be a recombinant peptide produced through molecular engineering techniques. The present section describes the methods and compositions involved in producing a composition of *Chlamydia psittaci* polypeptides for use as antigens in the present invention.

1. *Chlamydia psittaci* Polypeptides as Antigens

Section A describes methods for preparing a cloned *Chlamydia psittaci* library for ELI. Described also are methods for screening and identifying *Chlamydia psittaci* genes that confer protection against *Chlamydia psittaci* infection. Thus, *Chlamydia psittaci* polypeptide encoding genes or their corresponding cDNA identified in the present invention

can be inserted into an appropriate cloning vehicle for the production of *Chlamydia psittaci* polypeptides as antigens for the present invention. In addition, sequence variants of the polypeptide can be prepared. These may, for instance, be minor sequence variants of the polypeptide that arise due to natural variation within the population or they may be homologues found in other species. They also may be sequences that do not occur naturally, but that are sufficiently similar that they function similarly and/or elicit an immune response that cross-reacts with natural forms of the polypeptide. Sequence variants can be prepared by standard methods of site-directed mutagenesis such as those described below in the following section.

Another synthetic or recombinant variation of a *Chlamydia psittaci*-antigen is a polyepitopic moiety comprising repeats of epitopic determinants found naturally on *Chlamydia psittaci* proteins. Such synthetic polyepitopic proteins can be made up of several homomeric repeats of any one *Chlamydia psittaci* protein epitope; or can comprise of two or more heteromeric epitopes expressed on one or several *Chlamydia psittaci* protein epitopes.

Amino acid sequence variants of the polypeptide can be substitutional, insertional or deletion variants. Deletion variants lack one or more residues of the native protein which are not essential for function or immunogenic activity, and are exemplified by the variants lacking a transmembrane sequence described above. Another common type of deletion variant is one lacking secretory signal sequences or signal sequences directing a protein to bind to a particular part of a cell.

Substitutional variants typically contain the exchange of one amino acid for another at one or more sites within the protein, and may be designed to modulate one or more properties of the polypeptide such as stability against proteolytic cleavage. Substitutions preferably are conservative, that is, one amino acid is replaced with one of similar shape and charge. Conservative substitutions are well known in the art and include, for example, the changes of: alanine to serine; arginine to lysine; asparagine to glutamine or histidine; aspartate to glutamate; cysteine to serine; glutamine to asparagine; glutamate to aspartate;

glycine to proline; histidine to asparagine or glutamine; isoleucine to leucine or valine; leucine to valine or isoleucine; lysine to arginine; methionine to leucine or isoleucine; phenylalanine to tyrosine, leucine or methionine; serine to threonine; threonine to serine; tryptophan to tyrosine; tyrosine to tryptophan or phenylalanine; and valine to isoleucine or leucine.

Insertional variants include fusion proteins such as those used to allow rapid purification of the polypeptide and also can include hybrid proteins containing sequences from other proteins and polypeptides which are homologues of the polypeptide. For example, an insertional variant could include portions of the amino acid sequence of the polypeptide from one species, together with portions of the homologous polypeptide from another species. Other insertional variants can include those in which additional amino acids are introduced within the coding sequence of the polypeptide. These typically are smaller insertions than the fusion proteins described above and are introduced, for example, into a protease cleavage site.

In one embodiment, major antigenic determinants of the polypeptide may be identified by an empirical approach in which portions of the gene encoding the polypeptide are expressed in a recombinant host, and the resulting proteins tested for their ability to elicit an immune response. For example, the polymerase chain reaction (PCR) can be used to prepare a range of cDNAs encoding peptides lacking successively longer fragments of the C-terminus of the protein. The immunogenic activity of each of these peptides then identifies those fragments or domains of the polypeptide that are essential for this activity. Further experiments in which only a small number of amino acids are removed or added at each iteration then allows the location of other antigenic determinants of the polypeptide. Thus, the polymerase chain reaction, a technique for amplifying a specific segment of DNA *via* multiple cycles of denaturation-renaturation, using a thermostable DNA polymerase, deoxyribonucleotides and primer sequences is contemplated in the present invention (Mullis, 1990; Mullis *et al.*, 1992).

Another embodiment for the preparation of the polypeptides according to the invention is the use of peptide mimetics. Mimetics are peptide-containing molecules that mimic elements of protein secondary structure. Because many proteins exert their biological activity *via* relatively small regions of their folded surfaces, their actions can be reproduced by much smaller designer (mimetic) molecules that retain the bioactive surfaces and have potentially improved pharmacokinetic/dynamic properties (Fairlie *et al.*, 1998).

The underlying rationale behind the use of peptide mimetics is that the peptide backbone of proteins exists chiefly to orient amino acid side chains in such a way as to facilitate molecular interactions, such as those of antibody and antigen. However, unlike proteins, peptides often lack well defined three dimensional structure in aqueous solution and tend to be conformationally mobile. Progress has been made with the use of molecular constraints to stabilize the bioactive conformations. By affixing or incorporating templates that fix secondary and tertiary structures of small peptides, synthetic molecules (protein surface mimetics) can be devised to mimic the localized elements of protein structure that constitute bioactive surfaces. Methods for mimicking individual elements of secondary structure (helices, turns, strands, sheets) and for assembling their combinations into tertiary structures (helix bundles, multiple loops, helix-loop-helix motifs) have been reviewed (Fairlie *et al.*, 1998; Moore, 1994).

Methods for predicting, preparing, modifying, and screening mimetic peptides are described in U.S. Patent 5,933,819 and U.S. Patent 5,869,451 (each specifically incorporated herein by reference). It is contemplated in the present invention, that peptide mimetics will be useful in screening modulators of an immune response.

Modifications and changes may be made in the structure of a gene and still obtain a functional molecule that encodes a protein or polypeptide with desirable characteristics. The following is a discussion based upon changing the amino acids of a protein to create an equivalent, or even an improved, second-generation molecule. The amino acid changes may be achieved by changing the codons of the DNA sequence, according to the following data.

For example, certain amino acids may be substituted for other amino acids in a protein structure without appreciable loss of interactive binding capacity with structures such as, for example, antigen-binding regions of antibodies or binding sites on substrate molecules. Since it is the interactive capacity and nature of a protein that defines that protein's biological functional activity, certain amino acid substitutions can be made in a protein sequence, and its underlying DNA coding sequence, and nevertheless obtain a protein with like properties. It is thus contemplated by the inventor that various changes may be made in the DNA sequences of genes without appreciable loss of their biological utility or activity. Table 1 shows the codons that encode particular amino acids.

In making such changes, the hydropathic index of amino acids may be considered. The importance of the hydropathic amino acid index in conferring interactive biologic function on a protein is generally understood in the art (Kyte & Doolittle, 1982).

It is accepted that the relative hydropathic character of the amino acid contributes to the secondary structure of the resultant protein, which in turn defines the interaction of the protein with other molecules, for example, enzymes, substrates, receptors, DNA, antibodies, antigens, and the like.

Each amino acid has been assigned a hydropathic index on the basis of their hydrophobicity and charge characteristics (Kyte & Doolittle, 1982), these are: Isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophan (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5).

It is known in the art that certain amino acids may be substituted by other amino acids having a similar hydropathic index or score and still result in a protein with similar biological activity, *i.e.*, still obtain a biological functionally equivalent protein. In making such changes, the substitution of amino acids whose hydropathic indices are within ±2 is

preferred, those which are within ±1 are particularly preferred, and those within ±0.5 are even more particularly preferred.

It also is understood in the art that the substitution of like amino acids can be made effectively on the basis of hydrophilicity. U.S. Patent 4,554,101, incorporated herein by reference, states that the greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with a biological property of the protein.

As detailed in U.S. Patent 4,554,101, the following hydrophilicity values have been assigned to amino acid residues: arginine (+3.0); lysine (+3.0); aspartate (+3.0 ± 1); glutamate (+3.0 ± 1); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); threonine (-0.4); proline (-0.5 ± 1); alanine (-0.5); histidine *-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine (-1.8); isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5); tryptophan (-3.4).

It is understood that an amino acid can be substituted for another having a similar hydrophilicity value and still obtain a biologically equivalent and immunologically equivalent protein. In such changes, the substitution of amino acids whose hydrophilicity values are within ±2 is preferred, those that are within ±1 are particularly preferred, and those within ±0.5 are even more particularly preferred.

As outlined above, amino acid substitutions generally are based on the relative similarity of the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, and the like. Exemplary substitutions that take various of the foregoing characteristics into consideration are well known to those of skill in the art and include: arginine and lysine; glutamate and aspartate; serine and threonine; glutamine and asparagine; and valine, leucine and isoleucine.

2. Synthetic Polypeptides

Contemplated in the present invention are *Chlamydia psittaci* proteins and related peptides for use as antigens. In certain embodiments, the synthesis of a *Chlamydia psittaci* peptide fragment is considered. The peptides of the invention can be synthesized in solution or on a solid support in accordance with conventional techniques. Various automatic synthesizers are commercially available and can be used in accordance with known protocols. See, for example, Stewart and Young, (1984); Tam *et al.*, (1983); Merrifield, (1986); and Barany and Merrifield (1979), each incorporated herein by reference. Alternatively, recombinant DNA technology may be employed wherein a nucleotide sequence which encodes a peptide of the invention is inserted into an expression vector, transformed or transfected into an appropriate host cell and cultivated under conditions suitable for expression.

3. *Chlamydia psittaci* Polypeptide/Antigen Purification

Chlamydia psittaci polypeptides of the present invention are used as antigens for inducing a protective immune response in an animal and for the preparation of anti-*Chlamydia psittaci* antibodies. Thus, certain aspects of the present invention concern the purification, and in particular embodiments, the substantial purification, of a *Chlamydia psittaci* polypeptide that is described herein above. The term "purified protein or peptide " as used herein, is intended to refer to a composition, isolatable from other components, wherein the protein or peptide is purified to any degree relative to its naturally-obtainable state. A purified protein or peptide therefore also refers to a protein or peptide, free from the environment in which it may naturally occur.

Generally, "purified" will refer to a protein or peptide composition that has been subjected to fractionation to remove various other components, and which composition substantially retains its expressed biological activity. Where the term "substantially purified" is used, this designation will refer to a composition in which the protein or peptide forms the major component of the composition, such as constituting about 50% or more of the proteins in the composition.

Various methods for quantifying the degree of purification of the protein or peptide will be known to those of skill in the art in light of the present disclosure. These include, for example, determining the specific activity of an active fraction, or assessing the number of polypeptides within a fraction by SDS/PAGE analysis. A preferred method for assessing the purity of a fraction is to calculate the specific activity of the fraction, to compare it to the specific activity of the initial extract, and to thus calculate the degree of purity, herein assessed by a "-fold purification number." The actual units used to represent the amount of activity will, of course, be dependent upon the particular assay technique chosen to follow the purification and whether or not the expressed protein or peptide exhibits a detectable activity.

Various techniques suitable for use in protein purification will be well known to those of skill in the art. These include, for example, precipitation with ammonium sulphate, PEG, antibodies and the like or by heat denaturation, followed by centrifugation; chromatography steps such as ion exchange, gel filtration, reverse phase, hydroxylapatite and affinity chromatography; isoelectric focusing; gel electrophoresis; and combinations of such and other techniques. As is generally known in the art, it is believed that the order of conducting the various purification steps may be changed, or that certain steps may be omitted, and still result in a suitable method for the preparation of a substantially purified protein or peptide.

There is no general requirement that the protein or peptide always be provided in their most purified state. Indeed, it is contemplated that less substantially purified products will have utility in certain embodiments. Partial purification may be accomplished by using fewer purification steps in combination, or by utilizing different forms of the same general purification scheme. For example, it is appreciated that a cation-exchange column chromatography performed utilizing an HPLC apparatus will generally result in a greater-fold purification than the same technique utilizing a low pressure chromatography system. Methods exhibiting a lower degree of relative purification may have advantages in total recovery of protein product, or in maintaining the activity of an expressed protein.

It is known that the migration of a polypeptide can vary, sometimes significantly, with different conditions of SDS/PAGE (Capaldi *et al.*, 1977). It will therefore be appreciated that under differing electrophoresis conditions, the apparent molecular weights of purified or partially purified expression products may vary.

High Performance Liquid Chromatography (HPLC) is characterized by a very rapid separation with extraordinary resolution of peaks. This is achieved by the use of very fine particles and high pressure to maintain and adequate flow rate. Separation can be accomplished in a matter of minutes, or a most an hour. Moreover, only a very small volume of the sample is needed because the particles are so small and close-packed that the void volume is a very small fraction of the bed volume. Also, the concentration of the sample need not be very great because the bands are so narrow that there is very little dilution of the sample.

Gel chromatography, or molecular sieve chromatography, is a special type of partition chromatography that is based on molecular size. The theory behind gel chromatography is that the column, which is prepared with tiny particles of an inert substance that contain small pores, separates larger molecules from smaller molecules as they pass through or around the pores, depending on their size. As long as the material of which the particles are made does not adsorb the molecules, the sole factor determining rate of flow is the size. Hence, molecules are eluted from the column in decreasing size, so long as the shape is relatively constant. Gel chromatography is unsurpassed for separating molecules of different size because separation is independent of all other factors such as pH, ionic strength, temperature, *etc.* There also is virtually no adsorption, less zone spreading and the elution volume is related in a simple matter to molecular weight.

Affinity Chromatography is a chromatographic procedure that relies on the specific affinity between a substance to be isolated and a molecule that it can specifically bind to. This is a receptor-ligand type interaction. The column material is synthesized by

covalently coupling one of the binding partners to an insoluble matrix. The column material is then able to specifically adsorb the substance from the solution. Elution occurs by changing the conditions to those in which binding will not occur (alter pH, ionic strength, temperature, *etc.*).

5

D. Gene Delivery

In certain embodiments of the invention, an expression construct comprising a *Chlamydia psittaci* gene or polynucleotide segment under the control of a heterologous promoter operable in eukaryotic cells is provided. The general approach in certain aspects 10 of the present invention is to provide a cell with an expression construct encoding a specific *Chlamydia psittaci* protein, polypeptide or peptide fragment, thereby permitting the antigenic expression of the protein, polypeptide or peptide fragment to take effect in the cell. Following delivery of the expression construct, the protein, polypeptide or peptide fragment encoded by the expression construct is synthesized by the transcriptional and translational 15 machinery of the cell, as well as any that may be provided by the expression construct.

Viral and non-viral vector systems are the two predominate categories for the delivery of an expression construct encoding a therapeutic protein, polypeptide, polypeptide fragment. Both vector systems are described in the following sections. There also are two 20 primary approaches utilized in the delivery of an expression construct for the purposes of gene therapy; either indirect, *ex vivo* methods or direct, *in vivo* methods. *Ex vivo* gene transfer comprises vector modification of (host) cells in culture and the administration or transplantation of the vector modified cells to a gene therapy recipient. *In vivo* gene transfer comprises direct introduction of the vector (*e.g.*, injection, inhalation) into the target source 25 or therapeutic gene recipient.

In certain embodiments of the invention, the nucleic acid encoding the gene or polynucleotide may be stably integrated into the genome of the cell. In yet further embodiments, the nucleic acid may be stably or transiently maintained in the cell as a 30 separate, episomal segment of DNA. Such nucleic acid segments or "episomes" encode sequences sufficient to permit maintenance and replication independent of or in

synchronization with the host cell cycle. How the expression construct is delivered to a cell and/or where in the cell the nucleic acid remains is dependent on the type of vector employed. The following gene delivery methods provide the framework for choosing and developing the most appropriate gene delivery system for a preferred application.

5

1. Non-Viral Polynucleotide Delivery

In one embodiment of the invention, a polynucleotide expression construct consists of naked recombinant DNA or plasmids. In preferred embodiments of the invention, a *Chlamydia psittaci* polynucleotide is administered to a subject via injection 10 and/or particle bombardment (*e.g.*, a gene gun). Thus, in one preferred embodiment, polynucleotide expression constructs are transferred into cells by accelerating DNA-coated microprojectiles to a high velocity, allowing the DNA-coated microprojectiles to pierce cell membranes and enter cells. In another preferred embodiment, polynucleotides are administered to a subject by injection. Injection of a polynucleotide expression 15 construct may be given by intramuscular, intravenous, subcutaneous, ir intraperitoneal injection, as long as the polynucleotide expression construct can effectively be delivered to a desired target.

a. Particle Bombardment

20 Particle Bombardment depends on the ability to accelerate DNA-coated microprojectiles to a high velocity allowing them to pierce cell membranes and enter cells without killing them (Klein *et al.*, 1987). Several devices for accelerating small particles have been developed. The most commonly used forms rely on high-pressure helium gas (Sanford *et al.*, 1991), of which one of the present inventors is a co-inventor. 25 The microprojectiles used have consisted of biologically inert substances such as tungsten or gold beads.

For microprojectile bombardment transformation using the constructs of the instant invention, both physical and biological parameters may be optimized. Physical 30 factors are those that involve manipulating the DNA/microprojectile precipitate or those that affect the flight and velocity of either the macro- or microprojectiles. Biological

factors include all steps involved in manipulation of cells before and immediately after bombardment, such as the osmotic adjustment of target cells to help alleviate the trauma associated with bombardment, the orientation of an immature embryo or other target tissue relative to the particle trajectory, and also the nature of the transforming DNA, such as linearized DNA or intact supercoiled plasmids.

Accordingly, it is contemplated that one may wish to adjust various bombardment parameters in small scale studies to fully optimize the conditions. One may particularly wish to adjust physical parameters such as DNA concentration, gap distance, flight distance, tissue distance, and helium pressure. It is further contemplated that the grade of helium may effect transformation efficiency. One also may optimize the trauma reduction factors (TRFs) by modifying conditions which influence the physiological state of the recipient cells and which may therefore influence transformation and integration efficiencies. For example, the osmotic state, tissue hydration and the subculture stage or cell cycle of the recipient cells may be adjusted for optimum transformation.

Other physical factors include those that involve manipulating the DNA/microprojectile precipitate or those that affect the flight and velocity of either the macro- or microprojectiles. Biological factors include all steps involved in manipulation of cells immediately before and after bombardment. The pre-bombardment culturing conditions, such as osmotic environment, the bombardment parameters, and the plasmid configuration have been adjusted to yield the maximum numbers of stable transformants.

For microprojectile bombardment, one will attach (*i.e.*, "coat") DNA to the microprojectiles such that it is delivered to recipient cells in a form suitable for transformation thereof. In this respect, at least some of the transforming DNA must be available to the target cell for transformation to occur, while at the same time during delivery the DNA must be attached to the microprojectile. Therefore, availability of the transforming DNA from the microprojectile may comprise the physical reversal of interactions between transforming DNA and the microprojectile following delivery of the microprojectile to the target cell. This need not be the case, however, as availability to a

target cell may occur as a result of breakage of unbound segments of DNA or of other molecules which comprise the physical attachment to the microprojectile. Availability may further occur as a result of breakage of bonds between the transforming DNA and other molecules, which are either directly or indirectly attached to the microprojectile. It is further contemplated that transformation of a target cell may occur by way of direct illegitimate or homology-dependent recombination between the transforming DNA and the genomic DNA of the recipient cell. Therefore, as used herein, a "coated" microprojectile will be one which is capable of being used to transform a target cell, in that the transforming DNA will be delivered to the target cell, yet will be accessible to the target cell such that transformation may occur.

Any technique for coating microprojectiles which allows for delivery of transforming DNA to the target cells may be used. Methods for coating microprojectiles which have been demonstrated to work well with the current invention have been specifically disclosed herein. DNA may be bound to microprojectile particles using alternative techniques, however. For example, particles may be coated with streptavidin and DNA end labeled with long chain thiol cleavable biotinylated nucleotide chains. The DNA adheres to the particles due to the streptavidin-biotin interaction, but is released in the cell by reduction of the thiol linkage through reducing agents present in the cell.

Alternatively, particles may be prepared by functionalizing the surface of a gold oxide particle, providing free amine groups. DNA, having a strong negative charge, binds to the functionalized particles. Furthermore, charged particles may be deposited in controlled arrays on the surface of mylar flyer disks used in the PDS-1000 Biolistics device, thereby facilitating controlled distribution of particles delivered to target tissue.

b. Other Non-Viral Methods of Polynucleotide Delivery

Transfer of a cloned expression construct in the present invention also may be performed by any of the methods which physically or chemically permeabilize the cell membrane (e.g., calcium phosphate precipitation, DEAE-dextran, electroporation, direct microinjection, DNA-loaded liposomes and lipofectamine-DNA complexes, cell

sonication, gene bombardment using high velocity microprojectiles and receptor-mediated transfection.

In certain embodiments, the use of lipid formulations and/or nanocapsules is contemplated for the introduction of *a Chlamydia psittaci* polynucleotide or polypeptide, or a gene therapy vector into host cells.

Nanocapsules can generally entrap compounds in a stable and/or reproducible way. To avoid side effects due to intracellular polymeric overloading, such ultrafine particles (sized around 0.1 μm) should be designed using polymers able to be degraded *in vivo*. Biodegradable polyalkyl-cyanoacrylate nanoparticles that meet these requirements are contemplated for use in the present invention, and/or such particles may be easily made.

In a preferred embodiment of the invention, the polynucleotide or polypeptide may be associated with a lipid. The polynucleotide or polypeptide associated with a lipid may be encapsulated in the aqueous interior of a liposome, interspersed within the lipid bilayer of a liposome, attached to a liposome via a linking molecule that is associated with both the liposome and the oligonucleotide, entrapped in a liposome, complexed with a liposome, dispersed in a solution containing a lipid, mixed with a lipid, combined with a lipid, contained as a suspension in a lipid, contained or complexed with a micelle, or otherwise associated with a lipid. The lipid or lipid/ polynucleotide or polypeptide associated compositions of the present invention are not limited to any particular structure in solution. For example, they may be present in a bilayer structure, as micelles, or with a "collapsed" structure. They may also simply be interspersed in a solution, possibly forming aggregates which are not uniform in either size or shape.

Lipids suitable for use according to the present invention can be obtained from commercial sources. For example, dimyristyl phosphatidylcholine ("DMPC") can be obtained from Sigma Chemical Co., dicetyl phosphate ("DCP") is obtained from K & K

Laboratories (Plainview, NY); cholesterol ("Chol") is obtained from Calbiochem-Behring; dimyristyl phosphatidylglycerol ("DMPG") and other lipids may be obtained from Avanti Polar Lipids, Inc. (Birmingham, Ala.). Stock solutions of lipids in chloroform or chloroform/methanol can be stored at about -20°C. Preferably, chloroform is used as the only solvent since it is more readily evaporated than methanol.

"Liposome" is a generic term encompassing a variety of single and multilamellar lipid vehicles formed by the generation of enclosed lipid bilayers or aggregates. Liposomes may be characterized as having vesicular structures with a phospholipid bilayer membrane and an inner aqueous medium. Multilamellar liposomes have multiple lipid layers separated by aqueous medium. They form spontaneously when phospholipids are suspended in an excess of aqueous solution. The lipid components undergo self-rearrangement before the formation of closed structures and entrap water and dissolved solutes between the lipid bilayers (Ghosh and Bachhawat, 1991). However, the present invention also encompasses compositions that have different structures in solution than the normal vesicular structure. For example, the lipids may assume a micellar structure or merely exist as non-uniform aggregates of lipid molecules. Also contemplated are lipofectamine-nucleic acid complexes.

Liposomes within the scope of the present invention can be prepared in accordance with known laboratory procedures, for example: the method of Bangham *et al.* (1965), the contents of which are incorporated herein by reference; the method of Gregoriadis, as described in *DRUG CARRIERS IN BIOLOGY AND MEDICINE*, G. Gregoriadis ed. (1979) pp. 287-341, the contents of which are incorporated herein by reference; the method of Deamer and Uster (1983), the contents of which are incorporated by reference; and the reverse-phase evaporation method as described by Szoka and Papahadjopoulos (1978).

Other vector delivery systems which can be employed to deliver a nucleic acid encoding a therapeutic gene into cells are receptor-mediated delivery vehicles. These

take advantage of the selective uptake of macromolecules by receptor-mediated endocytosis in almost all eukaryotic cells. Because of the cell type-specific distribution of various receptors, the delivery can be highly specific (Wu and Wu, 1993).

Receptor-mediated gene targeting vehicles generally consist of two components: a cell receptor-specific ligand and a DNA-binding agent. Several ligands have been used for receptor-mediated gene transfer. The most extensively characterized ligands are asialoorosomucoid (ASOR) (Wu and Wu, 1987) and transferring (Wagner *et al.*, 1990). Recently, a synthetic neoglycoprotein, which recognizes the same receptor as ASOR, has been used as a gene delivery vehicle (Ferkol *et al.*, 1993; Perales *et al.*, 1994) and epidermal growth factor (EGF) has also been used to deliver genes to squamous carcinoma cells (Myers, EPO 0273085).

In other embodiments, the delivery vehicle may comprise a ligand and a liposome. For example, Nicolau *et al.* (1987) employed lactosyl-ceramide, a galactose-terminal asialganglioside, incorporated into liposomes and observed an increase in the uptake of the insulin gene by hepatocytes. Thus, it is feasible that a nucleic acid encoding a therapeutic gene also may be specifically delivered into a cell type such as prostate, epithelial or tumor endothelial cells, by any number of receptor-ligand systems with or without liposomes. For example, the human prostate-specific antigen (Watt *et al.*, 1986) may be used as the receptor for mediated delivery of a nucleic acid in prostate tissue.

In another embodiment of the invention, the expression construct may simply consist of naked recombinant DNA or plasmids. Transfer of the construct may be performed by any of the methods mentioned above which physically or chemically permeabilize the cell membrane. This is applicable particularly for transfer *in vitro*, however, it may be applied for *in vivo* use as well. Dubensky *et al.* (1984) successfully injected polyomavirus DNA in the form of $CaPO_4$ precipitates into liver and spleen of adult and newborn mice demonstrating active viral replication and acute infection. Benvenisty and Neshif (1986) also demonstrated that direct intraperitoneal injection of

$CaPO_4$ precipitated plasmids results in expression of the transfected genes. It is envisioned that DNA encoding a *Chlamydia psittaci* gene or polynucleotide of interest may also be transferred in a similar manner *in vivo* and express the gene or polynucleotide product.

5

2. Viral Vectors

In certain embodiments, it is contemplated that a *Chlamydia psittaci* gene or polynucleotide that confers immune resistance to infection may be delivered by a viral vector. The capacity of certain viral vectors to efficiently infect or enter cells, to
10 integrate into a host cell genome and stably express viral genes, have led to the development and application of a number of different viral vector systems (Robbins *et al.*, 1998). Viral systems are currently being developed for use as vectors for *ex vivo* and *in vivo* gene transfer. For example, adenovirus, herpes-simple virus, retrovirus and adeno-associated virus vectors are being evaluated currently for treatment of diseases
15 such as cancer, cystic fibrosis, Gaucher disease, renal disease and arthritis (Robbins and Ghivizzani, 1998; Imai *et al.*, 1998; U. S. Patent 5,670,488). The various viral vectors described below, present specific advantages and disadvantages, depending on the particular gene-therapeutic application.

20 #### a. Adenoviral Vectors

In particular embodiments, an adenoviral expression vector is contemplated for the delivery of expression constructs. "Adenovirus expression vector" is meant to include those constructs containing adenovirus sequences sufficient to (a) support packaging of the construct and (b) to ultimately express a tissue or cell-specific construct that has been
25 cloned therein.

Adenoviruses comprise linear double stranded DNA, with a genome ranging from 30 to 35 kb in size (Reddy *et al.*, 1998; Morrison *et al.*, 1997; Chillon *et al.*, 1999). An adenovirus expression vector according to the present invention comprises a genetically
30 engineered form of the adenovirus. Advantages of adenoviral gene transfer include the ability to infect a wide variety of cell types, including non-dividing cells, a mid-sized

genome, ease of manipulation, high infectivity and they can be grown to high titers (Wilson, 1996). Further, adenoviral infection of host cells does not result in chromosomal integration because adenoviral DNA can replicate in an episomal manner, without potential genotoxicity associated with other viral vectors. Adenoviruses also are structurally stable (Marienfeld *et al.*, 1999) and no genome rearrangement has been detected after extensive amplification (Parks *et al.*, 1997; Bett *et al.*, 1993).

Salient features of the adenovirus genome are an early region (E1, E2, E3 and E4 genes), an intermediate region (pIX gene, Iva2 gene), a late region (L1, L2, L3, L4 and L5 genes), a major late promoter (MLP), inverted-terminal-repeats (ITRs) and a ψ sequence (Zheng, *et al.*, 1999; Robbins *et al.*, 1998; Graham and Prevec, 1995). The early genes E1, E2, E3 and E4 are expressed from the virus after infection and encode polypeptides that regulate viral gene expression, cellular gene expression, viral replication, and inhibition of cellular apoptosis. Further on during viral infection, the MLP is activated, resulting in the expression of the late (L) genes, encoding polypeptides required for adenovirus encapsidation. The intermediate region encodes components of the adenoviral capsid. Adenoviral inverted terminal repeats (ITRs; 100-200 bp in length), are *cis* elements, function as origins of replication and are necessary for viral DNA replication. The ψ sequence is required for the packaging of the adenoviral genome.

A common approach for generating an adenoviruses for use as a gene transfer vector is the deletion of the E1 gene ($E1^-$), which is involved in the induction of the E2, E3 and E4 promoters (Graham and Prevec, 1995). Subsequently, a therapeutic gene or genes can be inserted recombinantly in place of the E1 gene, wherein expression of the therapeutic gene(s) is driven by the E1 promoter or a heterologous promoter. The $E1^-$, replication-deficient virus is then proliferated in a "helper" cell line that provides the E1 polypeptides *in trans* (e.g., the human embryonic kidney cell line 293). Thus, in the present invention it may be convenient to introduce the transforming construct at the position from which the E1-coding sequences have been removed. However, the position of insertion of the construct within the adenovirus sequences is not critical to the invention. Alternatively, the E3 region, portions of the E4 region or both may be deleted,

wherein a heterologous nucleic acid sequence under the control of a promoter operable in eukaryotic cells is inserted into the adenovirus genome for use in gene transfer (U. S. Patent 5,670,488; U. S. Patent 5,932,210, each specifically incorporated herein by reference).

Although adenovirus based vectors offer several unique advantages over other vector systems, they often are limited by vector immunogenicity, size constraints for insertion of recombinant genes and low levels of replication. The preparation of a recombinant adenovirus vector deleted of all open reading frames, comprising a full length dystrophin gene and the terminal repeats required for replication (Haecker *et al.*, 1997) offers some potentially promising advantages to the above mentioned adenoviral shortcomings. The vector was grown to high titer with a helper virus in 293 cells and was capable of efficiently transducing dystrophin in mdx mice, in myotubes *in vitro* and muscle fibers *in vivo*. Helper-dependent viral vectors are discussed below.

A major concern in using adenoviral vectors is the generation of a replication-competent virus during vector production in a packaging cell line or during gene therapy treatment of an individual. The generation of a replication-competent virus could pose serious threat of an unintended viral infection and pathological consequences for the patient. Armentano *et al.*, describe the preparation of a replication-defective adenovirus vector, claimed to eliminate the potential for the inadvertent generation of a replication-competent adenovirus (U. S. Patent 5,824,544, specifically incorporated herein by reference). The replication-defective adenovirus method comprises a deleted E1 region and a relocated protein IX gene, wherein the vector expresses a heterologous, mammalian gene.

Other than the requirement that the adenovirus vector be replication defective, or at least conditionally defective, the nature of the adenovirus vector is not believed to be crucial to the successful practice of the invention. The adenovirus may be of any of the 42 different known serotypes and/or subgroups A-F. Adenovirus type 5 of subgroup C is the preferred starting material in order to obtain the conditional replication-defective

adenovirus vector for use in the present invention. This is because adenovirus type 5 is a human adenovirus about which a great deal of biochemical and genetic information is known, and it has historically been used for most constructions employing adenovirus as a vector.

As stated above, the typical vector according to the present invention is replication defective and will not have an adenovirus E1 region. Adenovirus growth and manipulation is known to those of skill in the art, and exhibits broad host range *in vitro* and *in vivo* (U. S. Patent 5,670,488; U. S. Patent 5,932,210; U. S. Patent 5,824,54). This group of viruses can be obtained in high titers, *e.g.*, 10^9 to 10^{11} plaque-forming units per ml, and they are highly infective. The life cycle of adenovirus does not require integration into the host cell genome. The foreign genes delivered by adenovirus vectors are episomal and, therefore, have low genotoxicity to host cells. Many experiments, innovations, preclinical studies and clinical trials are currently under investigation for the use of adenoviruses as gene delivery vectors. For example, adenoviral gene delivery-based gene therapies are being developed for liver diseases (Han *et al.*, 1999), psychiatric diseases (Lesch, 1999), neurological diseases (Smith, 1998; Hermens and Verhaagen, 1998), coronary diseases (Feldman *et al.*, 1996), muscular diseases (Petrof, 1998), gastrointestinal diseases (Wu, 1998) and various cancers such as colorectal (Fujiwara and Tanaka, 1998; Dorai *et al.*, 1999), pancreatic (Carrion *et al.*, 1999), bladder (Irie *et al.*, 1999), head and neck (Blackwell *et al.*, 1999), breast (Stewart *et al.*, 1999), lung (Batra *et al.*, 1999) and ovarian (Vanderkwaak *et al.*, 1999).

b. Retroviral Vectors

In certain embodiments of the invention, the use of retroviruses for gene delivery are contemplated. Retroviruses are RNA viruses comprising an RNA genome. When a host cell is infected by a retrovirus, the genomic RNA is reverse transcribed into a DNA intermediate which is integrated into the chromosomal DNA of infected cells. This integrated DNA intermediate is referred to as a provirus. A particular advantage of retroviruses is that they can stably infect dividing cells with a gene of interest (*e.g.*, a therapeutic gene) by integrating into the host DNA, without expressing immunogenic viral proteins. Theoretically, the integrated retroviral vector will be maintained for the life of the infected host cell, expressing the gene of interest.

The retroviral genome and the proviral DNA have three genes: gag, pol, and env, which are flanked by two long terminal repeat (LTR) sequences. The gag gene encodes the internal structural (matrix, capsid, and nucleocapsid) proteins; the pol gene encodes the RNA-directed DNA polymerase (reverse transcriptase) and the env gene encodes viral envelope glycoproteins. The 5' and 3' LTRs serve to promote transcription and polyadenylation of the virion RNAs. The LTR contains all other cis-acting sequences necessary for viral replication.

A recombinant retrovirus of the present invention may be genetically modified in such a way that some of the structural, infectious genes of the native virus have been removed and replaced instead with a nucleic acid sequence to be delivered to a target cell (U. S. Patent 5,858,744; U. S. Patent 5,739,018, each incorporated herein by reference). After infection of a cell by the virus, the virus injects its nucleic acid into the cell and the retrovirus genetic material can integrate into the host cell genome. The transferred retrovirus genetic material is then transcribed and translated into proteins within the host cell. As with other viral vector systems, the generation of a replication-competent retrovirus during vector production or during therapy is a major concern. Retroviral vectors suitable for use in the present invention are generally defective retroviral vectors that are capable of infecting the target cell, reverse transcribing their RNA genomes, and integrating the reverse transcribed DNA into the target cell genome, but are incapable of

replicating within the target cell to produce infectious retroviral particles (e.g., the retroviral genome transferred into the target cell is defective in gag, the gene encoding virion structural proteins, and/or in pol, the gene encoding reverse transcriptase). Thus, transcription of the provirus and assembly into infectious virus occurs in the presence of an appropriate helper virus or in a cell line containing appropriate sequences enabling encapsidation without coincident production of a contaminating helper virus.

The growth and maintenance of retroviruses is known in the art (U. S. Patent 5,955,331; U. S. Patent 5,888,502, each specifically incorporated herein by reference). Nolan *et al.* describe the production of stable high titre, helper-free retrovirus comprising a heterologous gene (U. S. Patent 5,830,725, specifically incorporated herein by reference). Methods for constructing packaging cell lines useful for the generation of helper-free recombinant retroviruses with amphoteric or ecotrophic host ranges, as well as methods of using the recombinant retroviruses to introduce a gene of interest into eukaryotic cells *in vivo* and *in vitro* are contemplated in the present invention (U. S. Patent 5,955,331).

Currently, the majority of all clinical trials for vector mediated gene delivery use murine leukemia virus (MLV)-based retroviral vector gene delivery (Robbins *et al.*, 1998; Miller *et al.*, 1993). Disadvantages of retroviral gene delivery includes a requirement for ongoing cell division for stable infection and a coding capacity that prevents the delivery of large genes. However, recent development of vectors such as lentivirus (*e.g.*, HIV), simian immunodeficiency virus (SIV) and equine infectious-anemia virus (EIAV), which can infect certain non-dividing cells, potentially allow the *in vivo* use of retroviral vectors for gene therapy applications (Amado and Chen, 1999; Klimatcheva *et al.*, 1999; White *et al.*, 1999; Case et al., 1999). For example, HIV-based vectors have been used to infect non-dividing cells such as neurons (Takashi *et al.*, 1999; Miyake *et al.*, 1999), islets (Leibowitz *et al.*, 1999) and muscle cells (Johnston *et al.*, 1999). The therapeutic delivery of genes *via* retroviruses are currently being assessed for the treatment of various disorders such as inflammatory disease (Moldawer *et al.*; 1999),

AIDS (Amado *et al.*, 1999; Engel and Kohn, 1999), cancer (Clay *et al.*, 1999), cerebrovascular disease (Weihl *et al.*, 1999) and hemophilia (Kay, 1998).

c. Herpes-Simplex Viral Vectors

Herpes simplex virus (HSV) type I and type II contain a double-stranded, linear DNA genome of approximately 150 kb, encoding 70-80 genes. Wild type HSV are able to infect cells lytically and to establish latency in certain cell types (*e.g.*, neurons). Similar to adenovirus, HSV also can infect a variety of cell types including muscle (Yeung *et al.*, 1999), ear (Derby *et al.*, 1999), eye (Kaufman *et al.*, 1999), tumors (Yoon *et al.*, 1999; Howard *et al.*, 1999), lung (Kohut *et al.*, 1998), neuronal (Garrido et al., 1999; Lachmann and Efstathiou, 1999), liver (Miytake *et al.*, 1999; Kooby et al., 1999) and pancreatic islets (Rabinovitch *et al.*, 1999).

HSV viral genes are transcribed by cellular RNA polymerase II and are temporally regulated, resulting in the transcription and subsequent synthesis of gene products in roughly three discernable phases or kinetic classes. These phases of genes are referred to as the Immediate Early (IE) or alpha genes, Early (E) or beta genes and Late (L) or gamma genes. Immediately following the arrival of the genome of a virus in the nucleus of a newly infected cell, the IE genes are transcribed. The efficient expression of these genes does not require prior viral protein synthesis. The products of IE genes are required to activate transcription and regulate the remainder of the viral genome.

For use in therapeutic gene delivery, HSV must be rendered replication-defective. Protocols for generating replication-defective HSV helper virus-free cell lines have been described (U. S. Patent 5,879,934; U. S. Patent 5,851,826, each specifically incorporated herein by reference in its entirety). One IE protein, Infected Cell Polypeptide 4 (ICP4), also known as alpha 4 or Vmw175, is absolutely required for both virus infectivity and the transition from IE to later transcription. Thus, due to its complex, multifunctional nature and central role in the regulation of HSV gene expression, ICP4 has typically been the target of HSV genetic studies.

Phenotypic studies of HSV viruses deleted of ICP4 indicate that such viruses will be potentially useful for gene transfer purposes (Krisky *et al.*, 1998a). One property of viruses deleted for ICP4 that makes them desirable for gene transfer is that they only express the five other IE genes: ICP0, ICP6, ICP27, ICP22 and ICP47 (DeLuca *et al.*, 1985), without the expression of viral genes encoding proteins that direct viral DNA synthesis, as well as the structural proteins of the virus. This property is desirable for minimizing possible deleterious effects on host cell metabolism or an immune response following gene transfer. Further deletion of IE genes ICP22 and ICP27, in addition to ICP4, substantially improve reduction of HSV cytotoxicity and prevented early and late viral gene expression (Krisky *et al.*, 1998b).

The therapeutic potential of HSV in gene transfer has been demonstrated in various *in vitro* model systems and *in vivo* for diseases such as Parkinson's (Yamada *et al.*, 1999), retinoblastoma (Hayashi *et al.*, 1999), intracerebral and intradermal tumors (Moriuchi *et al.*, 1998), B cell malignancies (Suzuki *et al.*, 1998), ovarian cancer (Wang *et al.*, 1998) and Duchenne muscular dystrophy (Huard *et al.*, 1997).

d. Adeno-associated Viral Vectors

Adeno-associated virus (AAV), a member of the parvovirus family, is a human virus that is increasingly being used for gene delivery therapeutics. AAV has several advantageous features not found in other viral systems. First, AAV can infect a wide range of host cells, including non-dividing cells. Second, AAV can infect cells from different species. Third, AAV has not been associated with any human or animal disease and does not appear to alter the biological properties of the host cell upon integration. For example, it is estimated that 80-85% of the human population has been exposed to AAV. Finally, AAV is stable at a wide range of physical and chemical conditions which lends itself to production, storage and transportation requirements.

The AAV genome is a linear, single-stranded DNA molecule containing 4681 nucleotides. The AAV genome generally comprises an internal non-repeating genome

flanked on each end by inverted terminal repeats (ITRs) of approximately 145 bp in length. The ITRs have multiple functions, including origins of DNA replication, and as packaging signals for the viral genome. The internal non-repeated portion of the genome includes two large open reading frames, known as the AAV replication (rep) and capsid (cap) genes. The rep and cap genes code for viral proteins that allow the virus to replicate and package the viral genome into a virion. A family of at least four viral proteins are expressed from the AAV rep region, Rep 78, Rep 68, Rep 52, and Rep 40, named according to their apparent molecular weight. The AAV cap region encodes at least three proteins, VP1, VP2, and VP3.

AAV is a helper-dependent virus requiring co-infection with a helper virus (*e.g.*, adenovirus, herpesvirus or vaccinia) in order to form AAV virions. In the absence of co-infection with a helper virus, AAV establishes a latent state in which the viral genome inserts into a host cell chromosome, but infectious virions are not produced. Subsequent infection by a helper virus "rescues" the integrated genome, allowing it to replicate and package its genome into infectious AAV virions. Although AAV can infect cells from different species, the helper virus must be of the same species as the host cell (*e.g.*, human AAV will replicate in canine cells co-infected with a canine adenovirus).

AAV has been engineered to deliver genes of interest by deleting the internal non-repeating portion of the AAV genome and inserting a heterologous gene between the ITRs. The heterologous gene may be functionally linked to a heterologous promoter (constitutive, cell-specific, or inducible) capable of driving gene expression in target cells. To produce infectious recombinant AAV (rAAV) containing a heterologous gene, a suitable producer cell line is transfected with a rAAV vector containing a heterologous gene. The producer cell is concurrently transfected with a second plasmid harboring the AAV rep and cap genes under the control of their respective endogenous promoters or heterologous promoters. Finally, the producer cell is infected with a helper virus.

Once these factors come together, the heterologous gene is replicated and packaged as though it were a wild-type AAV genome. When target cells are infected with

the resulting rAAV virions, the heterologous gene enters and is expressed in the target cells. Because the target cells lack the rep and cap genes and the adenovirus helper genes, the rAAV cannot further replicate, package or form wild-type AAV.

The use of helper virus, however, presents a number of problems. First, the use of adenovirus in a rAAV production system causes the host cells to produce both rAAV and infectious adenovirus. The contaminating infectious adenovirus can be inactivated by heat treatment (56.degree. C. for 1 hour). Heat treatment, however, results in approximately a 50% drop in the titer of functional rAAV virions. Second, varying amounts of adenovirus proteins are present in these preparations. For example, approximately 50% or greater of the total protein obtained in such rAAV virion preparations is free adenovirus fiber protein. If not completely removed, these adenovirus proteins have the potential of eliciting an immune response from the patient. Third, AAV vector production methods which employ a helper virus require the use and manipulation of large amounts of high titer infectious helper virus, which presents a number of health and safety concerns, particularly in regard to the use of a herpesvirus. Fourth, concomitant production of helper virus particles in rAAV virion producing cells diverts large amounts of host cellular resources away from rAAV virion production, potentially resulting in lower rAAV virion yields.

e. Other Viral Vectors

The development and utility of viral vectors for gene delivery is constantly improving and evolving. Other viral vectors such as poxvirus; *e.g.*, vaccinia virus (Gnant *et al.*, 1999; Gnant *et al.*, 1999), alpha virus; *e.g.*, sindbis virus, Semliki forest virus (Lundstrom, 1999), reovirus (Coffey *et al.*, 1998) and influenza A virus (Neumann *et al.*, 1999) are contemplated for use in the present invention and may be selected according to the requisite properties of the target system.

In certain embodiments, vaccinia viral vectors are contemplated for use in the present invention. Vaccinia virus is a particularly useful eukaryotic viral vector system for expressing heterologous genes. For example, when recombinant vaccinia virus is

properly engineered, the proteins are synthesized, processed and transported to the plasma membrane. Vaccinia viruses as gene delivery vectors have recently been demonstrated to transfer genes to human tumor cells, *e.g.*, EMAP-II (Gnant *et al.*, 1999), inner ear (Derby *et al.*, 1999), glioma cells, *e.g.*, p53 (Timiryasova et al., 1999) and various mammalian cells, *e.g.*, P-450 (U. S. Patent 5,506,138). The preparation, growth and manipulation of vaccinia viruses are described in U. S. Patent 5,849,304 and U. S. Patent 5,506,138 (each specifically incorporated herein by reference).

In other embodiments, sindbis viral vectors are contemplated for use in gene delivery. Sindbis virus is a species of the alphavirus genus (Garoff and Li, 1998) which includes such important pathogens as Venezuelan, Western and Eastern equine encephalitis viruses (Sawai *et al.*, 1999; Mastrangelo *et al.*, 1999). *In vitro*, sindbis virus infects a variety of avian, mammalian, reptilian, and amphibian cells. The genome of sindbis virus consists of a single molecule of single-stranded RNA, 11,703 nucleotides in length. The genomic RNA is infectious, is capped at the 5' terminus and polyadenylated at the 3' terminus, and serves as mRNA. Translation of a vaccinia virus 26S mRNA produces a polyprotein that is cleaved co- and post-translationally by a combination of viral and presumably host-encoded proteases to give the three virus structural proteins, a capsid protein (C) and the two envelope glycoproteins (El and PE2, precursors of the virion E2).

Three features of sindbis virus suggest that it would be a useful vector for the expression of heterologous genes. First, its wide host range, both in nature and in the laboratory. Second, gene expression occurs in the cytoplasm of the host cell and is rapid and efficient. Third, temperature-sensitive mutations in RNA synthesis are available that may be used to modulate the expression of heterologous coding sequences by simply shifting cultures to the non-permissive temperature at various time after infection. The growth and maintenance of sindbis virus is known in the art (U. S. Patent 5,217,879, specifically incorporated herein by reference).

f. Chimeric Viral Vectors

Chimeric or hybrid viral vectors are being developed for use in therapeutic gene delivery and are contemplated for use in the present invention. Chimeric poxviral/retroviral vectors (Holzer *et al.*, 1999), adenoviral/retroviral vectors (Feng *et al.*, 1997; Bilbao *et al.*, 1997; Caplen *et al.*, 1999) and adenoviral/adeno-associated viral vectors (Fisher *et al.*, 1996; U. S. Patent 5,871,982) have been described.

These "chimeric" viral gene transfer systems can exploit the favorable features of two or more parent viral species. For example, Wilson *et al.*, provide a chimeric vector construct which comprises a portion of an adenovirus, AAV 5' and 3' ITR sequences and a selected transgene, described below (U. S. Patent 5,871,983, specifically incorporate herein by reference).

The adenovirus/AAV chimeric virus uses adenovirus nucleic acid sequences as a shuttle to deliver a recombinant AAV/transgene genome to a target cell. The adenovirus nucleic acid sequences employed in the hybrid vector can range from a minimum sequence amount, which requires the use of a helper virus to produce the hybrid virus particle, to only selected deletions of adenovirus genes, which deleted gene products can be supplied in the hybrid viral production process by a selected packaging cell. At a minimum, the adenovirus nucleic acid sequences employed in the pAdA shuttle vector are adenovirus genomic sequences from which all viral genes are deleted and which contain only those adenovirus sequences required for packaging adenoviral genomic DNA into a preformed capsid head. More specifically, the adenovirus sequences employed are the cis-acting 5' and 3' inverted terminal repeat (ITR) sequences of an adenovirus (which function as origins of replication) and the native 5' packaging/enhancer domain, that contains sequences necessary for packaging linear Ad genomes and enhancer elements for the E1 promoter. The adenovirus sequences may be modified to contain desired deletions, substitutions, or mutations, provided that the desired function is not eliminated.

The AAV sequences useful in the above chimeric vector are the viral sequences from which the rep and cap polypeptide encoding sequences are deleted. More specifically, the AAV sequences employed are the cis-acting 5' and 3' inverted terminal repeat (ITR) sequences. These chimeras are characterized by high titer transgene delivery to a host cell and the ability to stably integrate the transgene into the host cell chromosome (U. S. Patent 5,871,983, specifically incorporate herein by reference). In the hybrid vector construct, the AAV sequences are flanked by the selected adenovirus sequences discussed above. The 5' and 3' AAV ITR sequences themselves flank a selected transgene sequence and associated regulatory elements, described below. Thus, the sequence formed by the transgene and flanking 5' and 3' AAV sequences may be inserted at any deletion site in the adenovirus sequences of the vector. For example, the AAV sequences are desirably inserted at the site of the deleted E1a/E1b genes of the adenovirus. Alternatively, the AAV sequences may be inserted at an E3 deletion, E2a deletion, and so on. If only the adenovirus 5' ITR/packaging sequences and 3' ITR sequences are used in the hybrid virus, the AAV sequences are inserted between them.

The transgene sequence of the vector and recombinant virus can be a gene, a nucleic acid sequence or reverse transcript thereof, heterologous to the adenovirus sequence, which encodes a protein, polypeptide or peptide fragment of interest. The transgene is operatively linked to regulatory components in a manner which permits transgene transcription. The composition of the transgene sequence will depend upon the use to which the resulting hybrid vector will be put. For example, one type of transgene sequence includes a therapeutic gene which expresses a desired gene product in a host cell. These therapeutic genes or nucleic acid sequences typically encode products for administration and expression in a patient *in vivo* or *ex vivo* to replace or correct an inherited or non-inherited genetic defect or treat an epigenetic disorder or disease.

E. Antibodies Against *Chlamydia psittaci* Proteins.

In another aspect, the present invention provides antibody compositions that are immunoreactive with a *Chlamydia psittaci* polypeptide of the present invention, or any portion thereof.

An antibody can be a polyclonal or a monoclonal antibody. An antibody may also be monovalent or bivalent. A prototype antibody is an immunoglobulin composed by four polypeptide chains, two heavy and two light chains, held together by disulfide bonds. Each pair of heavy and light chains forms an antigen binding site, also defined as complementarity-determining region (CDR). Therefore, the prototype antibody has two CDRs, can bind two antigens, and because of this feature is defined bivalent. The prototype antibody can be split by a variety of biological or chemical means. Each half of the antibody can only bind one antigen and, therefore, is defined monovalent. Means for preparing and characterizing antibodies are well known in the art (see, *e.g.*, Howell and Lane, 1988).

Peptides corresponding to one or more antigenic determinants of a *Chlamydia psittaci* polypeptide of the present invention also can be prepared. Such peptides should generally be at least five or six amino acid residues in length, will preferably be about 10, 15, 20, 25 or about 30 amino acid residues in length, and may contain up to about 35-50 residues or so. Synthetic peptides will generally be about 35 residues long, which is the approximate upper length limit of automated peptide synthesis machines, such as those available from Applied Biosystems (Foster City, CA). Longer peptides also may be prepared, *e.g.*, by recombinant means.

The identification and preparation of epitopes from primary amino acid sequences on the basis of hydrophilicity is taught in U.S. Patent 4,554,101 (Hopp), incorporated herein by reference. Through the methods disclosed in Hopp, one of skill in the art would be able to identify epitopes from within an amino acid sequence such as a *Chlamydia psittaci* polypeptide sequence.

Numerous scientific publications have also been devoted to the prediction of secondary structure, and to the identification of epitopes, from analyses of amino acid sequences (Chou & Fasman, 1974a; Chou & Fasman, 1974b; Chou & Fasman, 1978a; Chou & Fasman, 1978b; Chou & Fasman, 1979). Any of these may be used, if desired, to supplement the teachings of Hopp in U.S. Patent 4,554,101.

Moreover, computer programs are currently available to assist with predicting antigenic portions and epitopic core regions of proteins. Examples include those programs based upon the Jameson-Wolf analysis (Jameson & Wolf, 1988; Wolf *et al.*, 1988), the program PEPPLOT® (Brutlag *et al.*, 1990; Weinberger *et al.*, 1985), and other new programs for protein tertiary structure prediction (Fetrow & Bryant, 1993). Another commercially available software program capable of carrying out such analyses is MACVECTOR (IBI, New Haven, CT).

In further embodiments, major antigenic determinants of a *Chlamydia psittaci* polypeptide may be identified by an empirical approach in which portions of the gene encoding the polypeptide are expressed in a recombinant host, and the resulting proteins tested for their ability to elicit an immune response. For example, PCR can be used to prepare a range of peptides lacking successively longer fragments of the C-terminus of the protein. The immunoactivity of each of these peptides is determined to identify those fragments or domains of the polypeptide that are immunodominant. Further studies in which only a small number of amino acids are removed at each iteration then allows the location of the antigenic determinants of the polypeptide to be more precisely determined.

Another method for determining the major antigenic determinants of a polypeptide is the SPOTS system (Genosys Biotechnologies, Inc., The Woodlands, TX). In this method, overlapping peptides are synthesized on a cellulose membrane, which following synthesis and deprotection, is screened using a polyclonal or monoclonal antibody. The antigenic determinants of the peptides which are initially identified can be further localized by performing subsequent syntheses of smaller peptides with larger

overlaps, and by eventually replacing individual amino acids at each position along the immunoreactive peptide.

Once one or more such analyses are completed, polypeptides are prepared that contain at least the essential features of one or more antigenic determinants. The peptides are then employed in the generation of antisera against the polypeptide. Minigenes or gene fusions encoding these determinants also can be constructed and inserted into expression vectors by standard methods, for example, using PCR cloning methodology.

The use of such small peptides for antibody generation or vaccination typically requires conjugation of the peptide to an immunogenic carrier protein, such as hepatitis B surface antigen, keyhole limpet hemocyanin or bovine serum albumin. Methods for performing this conjugation are well known in the art.

1. Anti-*Chlamydia psittaci* Antibody Generation

The present invention provides monoclonal antibody compositions that are immunoreactive with a *Chlamydia psittaci* polypeptide. As detailed above, in addition to antibodies generated against a full length *Chlamydia psittaci* polypeptide, antibodies also may be generated in response to smaller constructs comprising epitopic core regions, including wild-type and mutant epitopes. In other embodiments of the invention, the use of anti-*Chlamydia psittaci* single chain antibodies, chimeric antibodies, diabodies and the like are contemplated.

As used herein, the term "antibody" is intended to refer broadly to any immunologic binding agent such as IgG, IgM, IgA, IgD and IgE. Generally, IgG and/or IgM are preferred because they are the most common antibodies in the physiological situation and because they are most easily made in a laboratory setting.

Monoclonal antibodies (mAbs) are recognized to have certain advantages, *e.g.*, reproducibility and large-scale production, and their use is generally preferred.

However, "humanized" *Chlamydia psittaci* antibodies also are contemplated, as are chimeric antibodies from mouse, rat, goat or other species, fusion proteins, single chain antibodies, diabodies, bispecific antibodies, and other engineered antibodies and fragments thereof. As defined herein, a "humanized" antibody comprises constant regions from a human antibody gene and variable regions from a non-human antibody gene. A "chimeric antibody, comprises constant and variable regions from two genetically distinct individuals. An anti-*Chlamydia psittaci* humanized or chimeric antibody can be genetically engineered to comprise a *Chlamydia psittaci* antigen binding site of a given of molecular weight and biological lifetime, as long as the antibody retains its *Chlamydia psittaci* antigen binding site.

The term "antibody" is used to refer to any antibody-like molecule that has an antigen binding region, and includes antibody fragments such as Fab', Fab, $F(ab')_2$, single domain antibodies (DABs), Fv, scFv (single chain Fv), chimeras and the like. Methods and techniques of producing the above antibody-based constructs and fragments are well known in the art (U.S. Patent 5,889,157; U.S. Patent 5,821,333; U.S. Patent 5,888,773, each specifically incorporated herein by reference).

U.S. Patent 5,889,157 describes a humanized B3 scFv antibody preparation. The B3 scFv is encoded from a recombinant, fused DNA molecule, that comprises a DNA sequence encoding humanized Fv heavy and light chain regions of a B3 antibody and a DNA sequence that encodes an effector molecule. The effector molecule can be any agent having a particular biological activity which is to be directed to a particular target cell or molecule. Described in U.S. Patent 5,888,773, is the preparation of scFv antibodies produced in eukaryotic cells, wherein the scFv antibodies are secreted from the eukaryotic cells into the cell culture medium and retain their biological activity. It is contemplated that similar methods for preparing multi-functional anti-*Chlamydia psittaci* fusion proteins, as described above, may be utilized in the present invention.

Means for preparing and characterizing antibodies also are well known in the art (See, *e.g.*, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, 1988;

incorporated herein by reference). The methods for generating monoclonal antibodies (mAbs) generally begin along the same lines as those for preparing polyclonal antibodies. Briefly, a polyclonal antibody is prepared by immunizing an animal with an immunogenic *Chlamydia psittaci* polypeptide composition in accordance with the present invention and collecting antisera from that immunized animal.

A wide range of animal species can be used for the production of antisera. Typically the animal used for production of antisera is a rabbit, a mouse, a rat, a hamster, a guinea pig or a goat. Because of the relatively large blood volume of rabbits, a rabbit is a preferred choice for production of polyclonal antibodies.

As is well known in the art, a given composition may vary in its immunogenicity. It is often necessary therefore to boost the host immune system, as may be achieved by coupling a peptide or polypeptide immunogen to a carrier. Exemplary and preferred carriers are keyhole limpet hemocyanin (KLH) and bovine serum albumin (BSA). Other albumins such as ovalbumin, mouse serum albumin or rabbit serum albumin also can be used as carriers. Means for conjugating a polypeptide to a carrier protein are well known in the art and include glutaraldehyde, m-maleimidobenzoyl-N-hydroxysuccinimide ester, carbodiimide and bis-biazotized benzidine.

As also well known in the art, the immunogenicity of a particular immunogen composition can be enhanced by the use of non-specific stimulators of the immune response, known as adjuvants. Suitable molecule adjuvants include all acceptable immunostimulatory compounds, such as cytokines, toxins or synthetic compositions.

Adjuvants that may be used include IL-1, IL-2, IL-4, IL-7, IL-12, γ-interferon, GMCSP, BCG, aluminum hydroxide, MDP compounds, such as thur-MDP and nor-MDP, CGP (MTP-PE), lipid A, and monophosphoryl lipid A (MPL). RIBI, which contains three components extracted from bacteria, MPL, trehalose dimycolate (TDM) and cell wall skeleton (CWS) in a 2% squalene/Tween 80 emulsion also is contemplated. MHC antigens may even be used. Exemplary, often preferred adjuvants include

complete Freund's adjuvant (a non-specific stimulator of the immune response containing killed *Mycobacterium tuberculosis)*, incomplete Freund's adjuvants and aluminum hydroxide adjuvant.

In addition to adjuvants, it may be desirable to coadminister biologic response modifiers (BRM), which have been shown to upregulate T cell immunity or downregulate suppressor cell activity. Such BRMs include, but are not limited to, Cimetidine (CIM; 1200 mg/d) (SmithKline Beecham, PA); low-dose Cyclophosphamide (CYP; 300 mg/m^2) (Johnson/ Mead, NJ), cytokines such as γ-interferon, IL-2, or IL-12 or genes encoding proteins involved in immune helper functions, such as B-7.

The amount of immunogen composition used in the production of polyclonal antibodies varies upon the nature of the immunogen as well as the animal used for immunization. A variety of routes can be used to administer the immunogen (subcutaneous, intramuscular, intradermal, intravenous and intraperitoneal). The production of polyclonal antibodies may be monitored by sampling blood of the immunized animal at various points following immunization.

A second, booster injection, also may be given. The process of boosting and titering is repeated until a suitable titer is achieved. When a desired level of immunogenicity is obtained, the immunized animal can be bled and the serum isolated and stored, and/or the animal can be used to generate mAbs.

For production of rabbit polyclonal antibodies, the animal can be bled through an ear vein or alternatively by cardiac puncture. The removed blood is allowed to coagulate and then centrifuged to separate serum components from whole cells and blood clots. The serum may be used as is for various applications or else the desired antibody fraction may be purified by well-known methods, such as affinity chromatography using another antibody, a peptide bound to a solid matrix, or by using, *e.g.*, protein A or protein G chromatography.

mAbs may be readily prepared through use of well-known techniques, such as those exemplified in U.S. Patent 4,196,265, incorporated herein by reference. Typically, this technique involves immunizing a suitable animal with a selected immunogen composition, *e.g.*, a purified or partially purified *Chlamydia psittaci* polypeptide, peptide or domain, be it a wild-type or mutant composition. The immunizing composition is administered in a manner effective to stimulate antibody producing cells.

The methods for generating monoclonal antibodies (mAbs) generally begin along the same lines as those for preparing polyclonal antibodies. Rodents such as mice and rats are preferred animals, however, the use of rabbit, sheep or frog cells also is possible. The use of rats may provide certain advantages (Goding, 1986, pp. 60-61), but mice are preferred, with the BALB/c mouse being most preferred as this is most routinely used and generally gives a higher percentage of stable fusions.

The animals are injected with antigen, generally as described above. The antigen may be coupled to carrier molecules such as keyhole limpet hemocyanin if necessary. The antigen would typically be mixed with adjuvant, such as Freund's complete or incomplete adjuvant. Booster injections with the same antigen would occur at approximately two-week intervals, or the gene encoding the protein of interest can be directly injected.

Following immunization, somatic cells with the potential for producing antibodies, specifically B lymphocytes (B cells), are selected for use in the mAb generating protocol. These cells may be obtained from biopsied spleens, tonsils or lymph nodes, or from a peripheral blood sample. Spleen cells and peripheral blood cells are preferred, the former because they are a rich source of antibody-producing cells that are in the dividing plasmablast stage, and the latter because peripheral blood is easily accessible.

Often, a panel of animals will have been immunized and the spleen of an animal with the highest antibody titer will be removed and the spleen lymphocytes obtained by

homogenizing the spleen with a syringe. Typically, a spleen from an immunized mouse contains approximately 5×10^7 to 2×10^8 lymphocytes.

The antibody-producing B lymphocytes from the immunized animal are then fused with cells of an immortal myeloma cell, generally one of the same species as the animal that was immunized. Myeloma cell lines suited for use in hybridoma-producing fusion procedures preferably are non-antibody-producing, have high fusion efficiency, and enzyme deficiencies that render then incapable of growing in certain selective media which support the growth of only the desired fused cells (hybridomas).

Any one of a number of myeloma cells may be used, as are known to those of skill in the art (Goding, pp. 65-66, 1986; Campbell, pp. 75-83, 1984). For example, where the immunized animal is a mouse, one may use P3-X63/Ag8, X63-Ag8.653, NS1/1.Ag 4 1, Sp210-Ag14, FO, NSO/U, MPC-11, MPC11-X45-GTG 1.7 and S194/5XX0 Bul; for rats, one may use R210.RCY3, Y3-Ag 1.2.3, IR983F and 4B210; and U-266, GM1500-GRG2, LICR-LON-HMy2 and UC729-6 are all useful in connection with human cell fusions.

One preferred murine myeloma cell is the NS-1 myeloma cell line (also termed P3-NS-1-Ag4-1), which is readily available from the NIGMS Human Genetic Mutant Cell Repository by requesting cell line repository number GM3573. Another mouse myeloma cell line that may be used is the 8-azaguanine-resistant mouse murine myeloma SP2/0 non-producer cell line.

Methods for generating hybrids of antibody-producing spleen or lymph node cells and myeloma cells usually comprise mixing somatic cells with myeloma cells in a 2:1 proportion, though the proportion may vary from about 20:1 to about 1:1, respectively, in the presence of an agent or agents (chemical or electrical) that promote the fusion of cell membranes. Fusion methods using Sendai virus have been described by Kohler and Milstein (1975; 1976), and those using polyethylene glycol (PEG), such as 37% (v/v)

PEG, by Gefter *et al.* (1977). The use of electrically induced fusion methods also is appropriate (Goding pp. 71-74, 1986).

Fusion procedures usually produce viable hybrids at low frequencies, about 1×10^{-6} to 1×10^{-8}. However, this does not pose a problem, as the viable, fused hybrids are differentiated from the parental, unfused cells (particularly the unfused myeloma cells that would normally continue to divide indefinitely) by culturing in a selective medium. The selective medium is generally one that contains an agent that blocks the *de novo* synthesis of nucleotides in the tissue culture media. Exemplary and preferred agents are aminopterin, methotrexate, and azaserine. HAT medium, a growth medium containing hypoxanthine, aminopterin and thymidine, is well known in the art as a medium for selection of hybrid cells. Aminopterin and methotrexate block *de novo* synthesis of both purines and pyrimidines, whereas azaserine blocks only purine synthesis. Where aminopterin or methotrexate is used, the media is supplemented with hypoxanthine and thymidine as a source of nucleotides (HAT medium). Where azaserine is used, the media is supplemented with hypoxanthine.

The preferred selection medium is HAT. Only cells capable of operating nucleotide salvage pathways are able to survive in HAT medium. The myeloma cells are defective in key enzymes of the salvage pathway, *e.g.*, hypoxanthine phosphoribosyl transferase (HPRT), and they cannot survive. The B cells can operate this pathway, but they have a limited life span in culture and generally die within about two weeks. Therefore, the only cells that can survive in the selective media are those hybrids formed from myeloma and B cells.

This culturing provides a population of hybridomas from which specific hybridomas are selected. Typically, selection of hybridomas is performed by culturing the cells by single-clone dilution in microtiter plates, followed by testing the individual clonal supernatants (after about two to three weeks) for the desired reactivity. The assay should be sensitive, simple and rapid, such as radioimmunoassays, enzyme

immunoassays, cytotoxicity assays, plaque assays, dot immunobinding assays, and the like.

The selected hybridomas then would be serially diluted and cloned into individual antibody-producing cell lines, which clones can then be propagated indefinitely to provide mAbs. The cell lines may be exploited for mAb production in two basic ways. First, a sample of the hybridoma can be injected (often into the peritoneal cavity) into a histocompatible animal of the type that was used to provide the somatic and myeloma cells for the original fusion (*e.g.*, a syngeneic mouse). Optionally, the animals are primed with a hydrocarbon, especially oils such as pristane (tetramethylpentadecane) prior to injection. The injected animal develops tumors secreting the specific monoclonal antibody produced by the fused cell hybrid. The body fluids of the animal, such as serum or ascites fluid, can then be tapped to provide mAbs in high concentration. Second, the individual cell lines could be cultured *in vitro*, where the mAbs are naturally secreted into the culture medium from which they can be readily obtained in high concentrations.

mAbs produced by either means may be further purified, if desired, using filtration, centrifugation and various chromatographic methods such as HPLC or affinity chromatography. Fragments of the monoclonal antibodies of the invention can be obtained from the monoclonal antibodies so produced by methods which include digestion with enzymes, such as pepsin or papain, and/or by cleavage of disulfide bonds by chemical reduction. Alternatively, monoclonal antibody fragments encompassed by the present invention can be synthesized using an automated peptide synthesizer.

It also is contemplated that a molecular cloning approach may be used to generate monoclonals. For this, combinatorial immunoglobulin phagemid libraries are prepared from RNA isolated from the spleen of the immunized animal, and phagemids expressing appropriate antibodies are selected by panning using cells expressing the antigen and control cells. The advantages of this approach over conventional hybridoma techniques are that approximately 10^4 times as many antibodies can be produced and screened in a

single round, and that new specificities are generated by H and L chain combination which further increases the chance of finding appropriate antibodies.

Alternatively, monoclonal antibody fragments encompassed by the present invention can be synthesized using an automated peptide synthesizer, or by expression of full-length gene or of gene fragments in, for example, *E. coli*.

F. Pharmaceutical Compositions

Aqueous compositions of the present invention comprise an effective amount of a purified polynucleotide comprising a *Chlamydia psittaci* sequence and/or a purified a protein, polypeptide, peptide, epitopic core region of a *Chlamydia psittaci* protein, and the like, dissolved and/or dispersed in a pharmaceutically acceptable carrier and/or aqueous medium. Aqueous compositions of gene therapy vectors expressing any of the foregoing are also contemplated.

The phrases "pharmaceutically and/or pharmacologically acceptable" refer to molecular entities and/or compositions that do not produce an adverse, allergic and/or other untoward reaction when administered to an animal.

As used herein, "pharmaceutically acceptable carrier" includes any and/or all solvents, dispersion media, coatings, antibacterial and/or antifungal agents, isotonic and/or absorption delaying agents and the like. The use of such media and/or agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media and/or agent is incompatible with the active ingredient, its use in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions. For animal and more particularly human administration, preparations should meet sterility, pyrogenicity, general safety and/or purity standards as required by FDA Office of Biologics standards.

The biological material should be extensively dialyzed to remove undesired small molecular weight molecules and/or lyophilized for more ready formulation into a desired

vehicle, where appropriate. The active compounds may generally be formulated for parenteral administration, *e.g.*, formulated for injection via the intravenous, intramuscular, sub-cutaneous, intralesional, and/or even intraperitoneal routes, or formulated for oral or inhaled delivery. The preparation of an aqueous compositions that contain an effective amount of purified *Chlamydia psittaci* polynucleotide or polypeptide agent as an active component and/or ingredient will be known to those of skill in the art in light of the present disclosure. Typically, such compositions can be prepared as injectables, either as liquid solutions and/or suspensions; solid forms suitable for using to prepare solutions and/or suspensions upon the addition of a liquid prior to injection can also be prepared; and/or the preparations can also be emulsified.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions and/or dispersions; formulations including sesame oil, peanut oil and/or aqueous propylene glycol; and/or sterile powders for the extemporaneous preparation of sterile injectable solutions and/or dispersions. In all cases the form must be sterile and/or must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and/or storage and/or must be preserved against the contaminating action of microorganisms, such as bacteria and/or fungi.

Solutions of the active compounds as free base and/or pharmacologically acceptable salts can be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and/or mixtures thereof and/or in oils. Under ordinary conditions of storage and/or use, these preparations contain a preservative to prevent the growth of microorganisms.

A *Chlamydia psittaci* polynucleotide or polypeptide of the present invention can be formulated into a composition in a neutral and/or salt form. Pharmaceutically acceptable salts, include the acid addition salts (formed with the free amino groups of the protein) and/or which are formed with inorganic acids such as, for example, hydrochloric and/or phosphoric acids, and/or such organic acids as acetic, oxalic, tartaric, mandelic,

and/or the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, and/or ferric hydroxides, and/or such organic bases as isopropylamine, trimethylamine, histidine, procaine and/or the like. In terms of using peptide therapeutics as active ingredients, the technology of U.S. Patents 4,608,251; 4,601,903; 4,599,231; 4,599,230; 4,596,792; and/or 4,578,770, each incorporated herein by reference, may be used.

The carrier can also be a solvent and/or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and/or liquid polyethylene glycol, and/or the like), suitable mixtures thereof, and/or vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and/or by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and/or antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and/or the like. In many cases, it will be preferable to include isotonic agents, for example, sugars and/or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and/or gelatin.

Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and/or the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and/or freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. The preparation of more, and/or highly, concentrated solutions for direct injection is also contemplated, where the use of DMSO as solvent is envisioned to result in extremely

rapid penetration, delivering high concentrations of the active agents to a small tumor area.

Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and/or in such amount as is therapeutically effective. The formulations are easily administered in a variety of dosage forms, such as the type of injectable solutions described above, but drug release capsules and/or the like can also be employed.

For parenteral administration in an aqueous solution, for example, the solution should be suitably buffered if necessary and/or the liquid diluent first rendered isotonic with sufficient saline and/or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and/or intraperitoneal administration. In this connection, sterile aqueous media which can be employed will be known to those of skill in the art in light of the present disclosure. For example, one dosage could be dissolved in 1 ml of isotonic NaCl solution and/or either added to 1000 ml of hypodermoclysis fluid and/or injected at the proposed site of infusion, (see for example, "Remington's Pharmaceutical Sciences" 15th Edition, pages 1035-1038 and/or 1570-1580). Some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject.

A *Chlamydia psittaci* polynucleotide or protein-derived peptides and/or agents may be formulated within a therapeutic mixture to comprise about 0.0001 to 1.0 milligrams, and/or about 0.001 to 0.1 milligrams, and/or about 0.1 to 1.0 and/or even about 10 milligrams per dose and/or so. Multiple doses can also be administered.

In addition to the compounds formulated for parenteral administration, such as intravenous and/or intramuscular injection, other pharmaceutically acceptable forms include, *e.g.*, tablets and/or other solids for oral administration; liposomal formulations; time release capsules; and/or any other form currently used, including cremes.

One may also use nasal solutions and/or sprays, aerosols and/or inhalants in the present invention. Nasal solutions are usually aqueous solutions designed to be administered to the nasal passages in drops and/or sprays. Nasal solutions are prepared so that they are similar in many respects to nasal secretions, so that normal ciliary action is maintained. Thus, the aqueous nasal solutions usually are isotonic and/or slightly buffered to maintain a pH of 5.5 to 6.5. In addition, antimicrobial preservatives, similar to those used in ophthalmic preparations, and/or appropriate drug stabilizers, if required, may be included in the formulation. Various commercial nasal preparations are known and/or include, for example, antibiotics and/or antihistamines and/or are used for asthma prophylaxis.

Additional formulations which are suitable for other modes of administration include vaginal suppositories and/or pessaries. A rectal pessary and/or suppository may also be used. Suppositories are solid dosage forms of various weights and/or shapes, usually medicated, for insertion into the rectum, vagina and/or the urethra. After insertion, suppositories soften, melt and/or dissolve in the cavity fluids. In general, for suppositories, traditional binders and/or carriers may include, for example, polyalkylene glycols and/or triglycerides; such suppositories may be formed from mixtures containing the active ingredient in the range of 0.5% to 10%, preferably 1%-2%.

Oral formulations include such normally employed excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate and/or the like. These compositions take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations and/or powders. In certain defined embodiments, oral pharmaceutical compositions will comprise an inert diluent and/or assimilable edible carrier, and/or they may be enclosed in hard and/or soft shell gelatin capsule, and/or they may be compressed into tablets, and/or they may be incorporated directly with the food of the diet. For oral therapeutic administration, the active compounds may be incorporated with excipients and/or used in the form of ingestible tablets, buccal tables, troches, capsules, elixirs, suspensions, syrups, wafers, and/or the like. Such compositions and/or preparations should contain at

least 0.1% of active compound. The percentage of the compositions and/or preparations may, of course, be varied and/or may conveniently be between about 2 to about 75% of the weight of the unit, and/or preferably between 25-60%. The amount of active compounds in such therapeutically useful compositions is such that a suitable dosage will be obtained.

The tablets, troches, pills, capsules and/or the like may also contain the following: a binder, as gum tragacanth, acacia, cornstarch, and/or gelatin; excipients, such as dicalcium phosphate; a disintegrating agent, such as corn starch, potato starch, alginic acid and/or the like; a lubricant, such as magnesium stearate; and/or a sweetening agent, such as sucrose, lactose and/or saccharin may be added and/or a flavoring agent, such as peppermint, oil of wintergreen, and/or cherry flavoring. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings and/or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, and/or capsules may be coated with shellac, sugar and/or both. A syrup of elixir may contain the active compounds sucrose as a sweetening agent methyl and/or propylparabens as preservatives, a dye and/or flavoring, such as cherry and/or orange flavor.

G. Kits

Therapeutic kits of the present invention are kits comprising a *Chlamydia psittaci* polynucleotide or polypeptide. Such kits will generally contain, in suitable container means, a pharmaceutically acceptable formulation of a *Chlamydia psittaci* polynucleotide or polypeptide or vector expressing any of the foregoing in a pharmaceutically acceptable formulation. The kit may have a single container means, and/or it may have distinct container means for each compound.

When the components of the kit are provided in one and/or more liquid solutions, the liquid solution is an aqueous solution, with a sterile aqueous solution being particularly preferred. The *Chlamydia psittaci* polynucleotide or polypeptide compositions may also be formulated into a syringeable composition. In which case, the

container means may itself be a syringe, pipette, and/or other such like apparatus; from which the formulation may be applied to an infected area of the body, injected into an animal, and/or even applied to and/or mixed with the other components of the kit.

However, the components of the kit may be provided as dried powder(s). When reagents and/or components are provided as a dry powder, the powder can be reconstituted by the addition of a suitable solvent. It is envisioned that the solvent may also be provided in another container means.

The container means will generally include at least one vial, test tube, flask, bottle, syringe and/or other container means, into which the *Chlamydia psittaci* polynucleotide or polypeptide formulation are placed, preferably, suitably allocated. The kits may also comprise a second container means for containing a sterile, pharmaceutically acceptable buffer and/or other diluent.

The kits of the present invention will also typically include a means for containing the vials in close confinement for commercial sale, such as, *e.g.*, injection and/or blow-molded plastic containers into which the desired vials are retained.

Irrespective of the number and/or type of containers, the kits of the invention may also comprise, and/or be packaged with, an instrument for assisting with the injection/administration and/or placement of the ultimate *Chlamydia psittaci* polynucleotide or polypeptide within the body of an animal. Such an instrument may be a syringe, pipette, forceps, and/or any such medically approved delivery vehicle.

H. Examples

The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the

present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

Example 1

Exemplary ELI Protocol

The following sections outline general methodology that one might use to prepare, screen and utilize ELI according to the present invention. Of course the following methods are merely general guidelines and should not limit one of skill in the art from modifying the present invention to accomplish a desired goal using ELI.

1. Library Construction

The present invention provides expression library constructs of genus *Chlamydia psittacii*. An expression library of *C. psittaci* can be produced by first physically shearing the genomic DNA of *C. psittaci* (*e.g., C. psittaci* strain B577) and size-selecting fragments of 300-800 base pairs. The protocol used by the present inventors to produce a *C. psittaci* library is similar to that described in Sykes and Johnston (1999). Adaptors were added and the DNA fragments ligated into a genetic immunization vector (FIG. 2) designed to link fragments to the mouse ubiquitin gene. However, the fragments can be blunt-end cloned.

This vector is known to enhance MHC class I-restricted immune responses (Sykes and Johnston, 1999), while sterilizing immunity against *Chlamydia* is thought to be MHC class II-dependent (Morrison *et al.*, 1995). However, any genetic immunization procedure, by the mechanism of intracellular expression of the inserted genes, will target towards class I antigen presentation. Nevertheless, both MHC class I- and class II-restricted immune responses to the expressed antigens are well documented (Barry *et al.*, 1995; Sykes and Johnston, 1999). The inventors observed, for instance, pronounced delayed-type hypersensitivity responses, mediated by MHC II-restricted $CD4^+$ Th1 cells, against protective *C. psittaci* B577 antigens, which were expressed from the ubiquitin fusion vector. In addition to the fact that MHC II-restricted immunity is generated by the

ubiquitin fusion vector, MHC I-restricted immunity appears to mediate protection in the early phase of chlamydial infection (Morrison et al., 1995; Rottenberg et al., 1999). This duality of the cellular immune response generated by the ubiquitin fusion vector might explain the efficacy of this vector for genetic immunization against intracellular bacteria.

A library of approximately 82,000 individual members was created and tested as 27 sub-libraries each with 2,400-3,400 plasmid clones. The average insert frequency was approximately 67% and the average insert size was 660 base pairs. Nitrocellulose replica filters were made of each original colony plating of a sub-library pool for subsequent retrieval of positive clones. This generated a library with approximately six-fold expression-equivalent redundancy. One expression equivalent is defined as the number of in-frame fragments necessary to completely represent all authentic open reading frames. Since the genome size of *C. psittaci* is approximately $1x10^6$ base pairs and only one-sixth of the actual open-reading frames will be cloned in the right orientation and frame, it requires at least six genomic equivalents to encode one expression equivalent. Each sub-library was propagated on plates and harvested to prepare DNA. DNA representing each sub-library was used for genetic immunization of mice in the following section.

2. Vaccination and Challenge

For the first round of testing, outbred, 6-week old, female NIH-Swiss Webster mice were inoculated with the purified DNA of each sub-library using both intramuscular (i.m.) and epidermal injection. The epidermal injection was effected with a gene gun (Sanford et al., 1991). Each mouse was given 50 µg DNA i.m. and 5 µg DNA by gene gun. It has been argued that the gene gun immunization favors a Th2 and the i.m. injection a Th1 type response (Feltquate et al., 1997), therefore both types of injection were given to each group. In the first round of testing, the prime inoculation was followed by a boost 9 weeks later, before intranasal challenge with 3×10^6 inclusion forming units (IFU) of *C. psittaci* strain B577 13 weeks after prime inoculation. The animals were sacrificed 12 days after the challenge, and lungs were weighed.

3. Library Deconvolution

The basic scheme for handling the reduction of the libraries is depicted in FIG. 3. Fourteen groups out of the first round looked promising, so the individual clones from these groups were picked and grown in 96 well microtiter plates. This gave approximately 40,000 wells in microtiter plates, therefore about 40,000 clones. The second round was reduced using a two dimensional array format. As depicted in FIG. 3, the DNA was prepared from colonies pooled from rows and columns of the array. The rationale was that if a row and column conferred protection, the colonies at the intersection would be responsible. This scheme is premised on largely additive effects of the protective clones. This 24x24 array yielded pools of ~1,700 clones with each intercession having ~96 clones. Currently the inventors deconvolute the second round with a 3-dimesional array.

Since the lung weight was highly variable in the outbred NIH-Swiss mice with variable MHC background, the inventors decided to use inbred BALB/c mice in subsequent rounds. The 48 DNA pools for round two were i.m. injected into BALB/c mice at 50 μg DNA/animal, and the animals were boosted at seven weeks by both gene gun inoculation and i.m. injection. The mice were given a higher *C. psittaci* challenge, 1.6×10^6 IFU *C. psittaci* B577, at approximately 12 weeks, again to further differentiate the groups. Animals were sacrificed and results evaluated as in round one.

In the fourth round, the animals received two boosts rather than one, and the challenge inoculum was increased to 3×10^6 IFU *C. psittaci* B577 to increase the selectivity of protection scoring. Furthermore, because too much DNA may lead to a decrease in cellular immune response, the amount of each individual clone was reduced by half, with the difference made up with pUC118 DNA, so each mouse received a total 50 μg DNA for i.m. immunization, but only 25 μg of the specific clone. The inventors also decreased the gene gun DNA in the same manner: 1.25 μg/ear of the specific clone and 1.25 μg pUC118. Mice were boosted i.m. at both four and nine weeks after prime inoculation, and were challenged. The results of this final round are depicted in FIG. 5.

4. Analysis of Sequences

The clones conferring protection were re-sequenced and then compared by BLAST search to Genbank and particularly to the recently completed *C. pneumoniae* (Kalman et al., 1999) genome sequences (FIG. 6). Of the 14 single genes identified in this study, ten are internal fragments and three contain the C-terminus of the protein. Of the five most protective clones, one was from a putative outer membrane protein and one was from a cell surface protein. The other three were from cytosolic proteins.

Four of the 14 clones have sequence similarity to a class of proteins known as putative outer membrane proteins (POMPs) in *Chlamydia psittaci* and *Chlamydia pneumoniae*. Many of the "putative" outer membrane proteins are known to be localized to the outer membrane and to be highly immunogenic (Longbottom et al., 1996; Tan et al., 1990).

5. Mixing Experiment

The two dimensional approach used to find protective gene fragments assumes that the protection is due to a single highly protective gene within a pool. To verify that such genes would be found, 25 ng (i.e. 1/2000) of either of the two most protective genes was added to a pool that scored negative (pool 6 round 1). As depicted in FIG. 7, spiking with either clone converted the negative library to a positive.

Example 2

Materials and Methods

Library construction. *C. psittaci* strain B577 (ATCC VR-656) was grown in BGMK cells and elementary bodies (EB) were purified by renograff gradient centrifugation as described (Huang et al., 1999). Genomic DNA was isolated from EB by proteinase K and RNase digestion followed by cetyl-trimethyl ammonium bromide (Kaltenbock et al., 1997).

Genomic DNA was physically sheared using a nebulizer (Glas Col, Terra Haute, IN), then size fractioned on a 1.5% TBE agarose gel. Agarose with fragments between

300-700 base pairs was excised and the DNA was electroeluted. Adaptors (top strand 5': GATCTGGATCCCGAT (SEQ ID NO.2) ATCGGGCTCCA (SEQ ID NO.3) onto the fragments, then the fragments were cloned into pCMVi-UBs at the Bgl II site (See FIG. 6 and Sykes and Johnston, 1999 for more details). The ligations were transformed into DH 5 alpha electrocompetent cells and plated onto 150 mm diameter YT-Ampicillin (75 µg/mL final concentration) plates. The resulting plates had between 2400-3400 individual clones per plate. After the plates were incubated overnight at 37°C, the colonies from were lifted using nitrocellulose filters soaked in L-Broth with 8% DMSO, and these filters were stored at –80 °C. The original agar plates were then incubated at 37 °C for an additional six hours. Ten mL of L Broth was added to each plate, the E. coli was scraped into 150 mL of L-Broth and grown at 37 °C for 30 minutes. Ampicillin was then added to a final concentration of 50 µg/mL, and the cultures were grown overnight at 37 °C. Cells were pelleted and the DNA was purified using Qiagen tip 500 columns.

Inoculation of DNA. Round One: DNA from the pools was injected into 6-week old female NIH-Swiss mice. All mice received 50 µg total DNA by i.m. injections, evenly distributed between the quadriceps and tibialis anterior muscles. Eighteen of the groups also received gene gun inoculations (wand), with 2.5 µg DNA inoculated into each ear. The animals were boosted once at nine weeks in the same manner as the primary inoculation – all mice received i.m. injections, but only the same 18 groups received gene gun injections -- then intranasally challenged with 5.5×10^5 IFU of *C. psittaci* strain B577 at 13 weeks. The mice were sacrificed 11 days after the challenge, and lungs were weighed.

Round Two: Nitrocellulose filters from the positive pools were placed on L-Broth Bio-Assay plates supplemented with 75 µg/mL ampicillin and 2% agar. The filters were incubated on the plates for approximately 15 minutes, then the nitrocellulose was discarded. The colonies were grown at 30°C for 12 hours. The majority of the colonies were picked into 96 well microtiter plates containing HYT media (1.6% Bacto-tryptone, 1.0% Bacto-yeast extract, 85.5 mM NaCl, 36 mM K_2HPO_4, 13.2 mM KH_2PO_4, 1.7 mM Sodium citrate, 0.4 mM MgSO4, 6.8 mM ammonium sulfate, 4.4 % wt/vol glycerol)

supplemented with 75 μg/mL ampicillin, using a Hybaid colony picker; the plates were then visually inspected and the remainder of the colonies were hand-picked. The microtiter plates were designated by their original pool number and by the order in which they were picked. Hence, plate 5.10 was from original pool 5 and was the tenth plate picked. The colonies were subdivided into groups as is indicated in FIG. 2. All of the microtiter plates comprising a pool were stamped onto on L-Broth Bio-Assay plates supplemented with 75 μg/mL ampicillin and were grown overnight at 37°C. The cells from these plates were harvested by adding L-Broth to the plates and scraping off the cells. The cells were pelleted by centrifugation then resuspended in Qiagen buffer P1. The remainder of the DNA prep proceeded according to manufacture's instructions.

These 48 DNA pools were i.m. injected into 6-week old BALB/c mice at 50 μg DNA/animal. For the initial inoculation, the mice did not receive gene gun inoculations. At seven weeks, the mice were boosted with 50 μg DNA/animal. In addition to the i.m. injections, the first 31 groups received gene gun (Rumsey-Loomis) inoculations at 2.5 μg DNA/ear; however, the gene gun failed at group 32, and the last 17 groups received only i.m. injections. The mice were given a higher challenge, 1.6×10^6 IFU *C. psittaci* B577, at 12 weeks. Animals were sacrificed as in round one.

Round Three: Colonies from the microtiter plates that were judged to be positive were arrayed as in FIG. 2. For each pool, new microtiter plates with HYT media supplemented with 75 μg/mL ampicillin were constructed from all of the colonies which comprise the. Colonies were grown and DNA prepared as in round two.

The mice received both gene gun (wand) and i.m. inoculations at the dosage indicated above. At six weeks, the mice were boosted with 50 μg DNA/animal, but only by i.m. injections. The challenge schedule was the same as in Round Two.

Round Four: *E. coli* from wells at either full by full protection or full by partial protection was streaked out onto YT-plates supplemented with 75 μg/mL ampicillin. Six colonies from each of the plates were tested by PCR colony screening, using the primers

FS-UB 5': CCGCACCCTCTCTGATTAC (SEQ ID No: 4) CTGGAGTGGCAACTTCC. (SEQ ID NO. 5) Colonies with different sizes, hence different inserts, were sequenced using ABI Big Dye terminator and the FS-UB primer. Samples were purified on G-50 spin columns, and run on an ABI 377 Sequencer. The generated sequences were analyzed for open reading frames using a program designed by Simon Raynor, Ph.D.

Example 3

Vaccination and Challenge

It was established that the weight increase of the infected lung over the lung weight of naïve, uninfected controls (~ 120 mg) correlated strongly with disease intensity. Maximum disease in this model resulted in approximately 250% lung weight increase, while further lung weight increases were lethal. The lung disease on day 12 after inoculation was characterized by areas of gross lung tissue consolidation and the presence of mononuclear interstitial infiltrates in consolidated tissue. Chlamydial inclusions were observed by immunohistochemistry in many macrophages, but rarely in other cells. Controls for complete protection were established by low level intranasal infection of naïve mice with 3×10^4 IFU of *C. psittaci* strain B577 4 weeks prior to challenge. These mice were completely protected from disease after challenge infection and had lung weight increases of 10-30% compared to naïve animals. Lungs of completely protected mice did not show gross lung lesions, and pathohistological examination revealed no interstitial infiltrates, but prominent peribronchiolar lymphocytic cuffs, interpreted as sign of protective immune stimulation. The chlamydial lung burden on day 11 after challenge was typically $1\text{-}3 \times 10^6$ IFU per 100mg lung tissue in protected, and $2\text{-}6 \times 10^5$ IFU per 100 mg lung in diseased animals. Since the lowest chlamydial burden was, however, not consistently associated with lowest disease, the inventors used the disease-dependent parameter lung weight rather than chlamydial burden as readout for evaluation of protection. The lung weights were transformed to relative protection scores in a linear equation that assumed the high average lung weight of the severely ill, naïve, challenged mice as 0 and that of fully protected controls as 1 (FIG. 4).

Example 4

Deconvolution of the Libraries

Since the lung weight was highly variable in the outbred NIH-Swiss mice with variable MHC background, the inventors decided to use inbred BALB/c mice in subsequent rounds. The 48 DNA pools for round two were i.m. injected into BALB/c mice at 50 μg DNA/animal, and the animals were boosted at seven weeks by both gene gun inoculation and i.m. injection. The mice were given a higher *C. psittaci* challenge, 1.6 x 10^6 IFU *C. psittaci* B577, at approximately 12 weeks, again to further differentiate the groups. Animals were sacrificed and results evaluated as in round one.

The results of the Round two challenge are depicted in FIG. 4. Of the 48 groups from round two, 15 were judged to be positive, giving a total of 3936 wells. These wells were again arrayed as in round two, but the array had 112 colonies per column and 156 per row with 4-5 colonies per intersection (See FIG. 3). The mice received both gene gun and i.m. injections at the dosage indicated above. At six weeks, the mice were boosted. Both the challenge and the sacrifice were performed as in Round two.

The positive 46 colonies from the intersection wells from Round three were sequenced, and those clones with open reading frames greater than 50 amino acids long were prepared individually and shot into mice as single genes and as a pool. Fourteen clones met these criteria. The disease scoring on each pool in rounds 1-3 are depicted in FIG. 4.

In the fourth round, the animals received two boosts rather than one, and the challenge inoculum was increased to 3 x10^6 IFU *C. psittaci* B577to increase the selectivity of protection scoring. Furthermore, because too much DNA may lead to a decrease in cellular immune response, the amount of each individual clone was reduced by half but made up the difference with pUC118 DNA, and each mouse received a total 50 μg DNA for i.m. immunization, but only 25 μg of the specific clone. The inventors also decreased the gene gun DNA in the same manner: 1.25 μg/ear of the specific clone

and 1.25 μg pUC118. Mice were boosted i.m. at both four and nine weeks after prime inoculation, and were challenged. The results of this final round are depicted in FIG. 5.

Example 5

Comparison of clones

Based on the hypothesis that sequences from genes conferring a high level of protection might be selected more than once in the ELI process, the clones were compared against each other for overlaps. Interestingly, one of the clones, CP4 #10, did overlap with another gene, CP4 #11. The gene from which these two clones arise had been partially sequenced (Longbottom et al., 1998).

Two of the genes, CP4 #5 and CP4 #9, had an overlapping region, but they were fused to ubiqutin in opposite orientations. CP4 #5, is composed of two different *C. psittaci* DNA fragments, fused in opposite orientations. The first gene is fused to ubiqutin in the correct orientation and the correct reading frame. Interestingly, the second gene, which is in the opposite orientation to the ubiqutin gene, has an overlapping sequence to CP4 #5. It is doubtful that the protein from the second gene is produced in the mouse.

Example 6

Analysis of Sequences

The clones conferring protection were re-sequenced and then compared by BLAST search to Genbank and particularly to the recently completed *C. pneumoniae* (Kalman et al., 1999) genome sequences (FIG. 6). The full-length *Chlamydia psittaci* genes were next isolated and sequences. Upon analysis, all nucleic acid sequences, except #4, #10, #11, and #12, were previously undisclosed in any context. Further, only protions of the sequences encoding #10 and #11 were previously disclosed.

Since most protective genes would not have been predicted by any bioinformatics or information-based approach, it is likely that one will need to apply an unbiased, global approach, such as ELI to define vaccine candidates.

Table 2, lists a comparision of the *Chlamydia psittaci* genes with homologues from *Chlamydia trachomatis* and *Chlamydia pneumoniae*.

Table 2

	C. psittaci	*C. trachomatis*	Identity/ Similarity	*C. pneumoniae*	Identity/ Similarity
CP4 #1		DNA Pol III Gamma and Tau	62/73	DNA Pol III Gamma and Tau	66/76
CP4 #2		Glu-tRNA Gln Amido-transferase (C subunit)	49/70	Glu-tRNA Gln Amido-transferase (C subunit)	48/63
CP4 #3		Glu-tRNA Gln Amido-transferase (A subunit)	71/85	Glu-tRNA Gln Amido-transferase (A subunit)	71/84
CP4 #4	OMP 90A	Outer Membrane Protein 5	30/45	Outer Membrane Protein G Fami Outer Membrane Protein G/I Fa	40/54 28/46
CP4 #5		Transglycolase/transpeptida	67/80	Transglycolase/transpeptidase	67/77
CP4 #6		Protein Translocase	80/89	Protein Translocase	84/92
CP4 #7				Outer Membrane Lipoprotein	60/79
CP4 #8		Oligopeptidase	60/75	Oligopeptidase	61/74
CP4 #9		Hypothetical protein	62/76	Hypothetical protein	62/77
CP4 #10		Outer Membrane Protein 4	27/42	Outer Membrane Protein G fami	33/51
CP4 #11		Outer Membrane Protein 4	27/42	Outer Membrane Protein G famil	33/51
CP4 #12	OMP 98 kDa	Outer Membrane Protein 5	30/43	Outer membrane Protein G famil	44/58
CP4 #13		Protein Translocase	80/89	Protein Translocase	84/92
CP4 #14		Succinate Dehydrogenase	60/76	Succinate Dehydrogenase	61/77

Table 3 lists all of the cloned fragments, their corresponsing full length nucleotide sequences, and the amino acid sequences encoded by both the fragments and the full length sequences. Table 2 further describes the fragments.

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TABLE 3—SEQUENCE LISTING INDEX

SEQ ID NO	CP4_NO	Description
SEQ ID NO:6	CP4 #1	(fragment) homolog to *C. pneumoniae* DNA Pol III Gamma and Tau subunits (dnaX2 gene)
SEQ ID NO:7	CP4 #1	Polypeptide translation corresponding to SEQ ID NO. 6, homolog to *C. pneumoniae* DNA Pol III Gamma and Tau subunits (dnaX2 gene)
SEQ ID NO:8	CP4 #1	(full length) homolog to *C. pneumoniae* DNA Pol III Gamma and Tau subunits (dnaX2 gene)
SEQ ID NO:9	CP4 #1	Polypeptide translation corresponding to SEQ ID NO. 8, homolog to *C. pneumoniae* DNA Pol III Gamma and Tau subunits (dnaX2 gene)
SEQ ID NO:10	CP4 #2	(fragment) homolog to *C. pneumoniae* Glu-tRNA Gln Amido-transferase (C subunit) (gatC gene)
SEQ ID NO:11	CP4 #2	Polypeptide translation corresponding to SEQ ID NO. 10, homolog to *C. pneumoniae* Glu-tRNA Gln Amido-transferase (C subunit) (gatC gene)
SEQ ID NO:12	CP4 #2	(full length) homolog to *C. pneumoniae* Glu-tRNA Gln Amido-transferase (C subunit) (gatC gene)
SEQ ID NO:13	CP4 #2	Polypeptide translation corresponding to SEQ ID NO. 12, homolog to *C. pneumoniae* Glu-tRNA Gln Amido-transferase (C subunit) (gatC gene)
SEQ ID NO:14	CP4 #3	(fragment) homolog to *C. pneumoniae* Glu-tRNA Gln Amido-transferase (A subunit) (gatA gene)
SEQ ID NO:15	CP4 #3	Polypeptide translation corresponding to SEQ ID NO. 14, homolog to *C. pneumoniae* Glu-tRNA Gln Amido-transferase (A subunit) (gatA gene)

SEQ ID NO:16	CP4 #3	(full length) homolog to *C. pneumoniae* Glu-tRNA Gln Amido-transferase (A subunit) (gatA gene)
SEQ ID NO:17	CP4 #3	Polypeptide translation corresponding to SEQ ID NO. 16, homolog to *C. pneumoniae* Glu-tRNA Gln Amido-transferase (A subunit) (gatA gene)
SEQ ID NO:18	CP4 #3	(full length) homolog to *C. pneumoniae* Glu-tRNA Gln Amido-transferase (B subunit) (gatB gene)
SEQ ID NO:19	CP4 #3	Polypeptide translation corresponding to SEQ ID NO. 18, homolog to *C. pneumoniae* Glu-Trna Gln Amido-transferase (B subunit) (gatB gene)
SEQ ID NO:20	CP4 #4	(fragment) *C. psittaci* 90 kDa outer membrane protein (OMP90A gene) (Previously sequenced by Longbottom, et al), homolog to *Chlamydia pneumoniae* Outer Membrane Protein G/I (pmp 9) and Outer Membrane Protein G (pmp 5)
SEQ ID NO:21	CP4 #4	Polypeptide translation corresponding to SEQ ID NO. 20, *C. psittaci* 90 kDa outer membrane protein (OMP90A gene), homolog to *Chlamydia pneumoniae* Outer Membrane Protein G/I (pmp 9) and Outer Membrane Protein G (pmp 5)
SEQ ID NO:22	CP4 #4	(full length) *C. psittaci* 90 kDa outer membrane protein (OMP90A gene) (Previously sequenced by Longbottom, et al), homolog to *Chlamydia pneumoniae* Outer Membrane Protein G/I (pmp 9) and Outer Membrane Protein G (pmp 5)
SEQ ID NO:23	CP4 #4	Polypeptide translation corresponding to SEQ ID NO. 22, *C. psittaci* 90 kDa outer membrane protein (OMP90A gene), homolog to *Chlamydia pneumoniae* Outer Membrane Protein G/I (pmp 9) and Outer Membrane Protein G (pmp 5)
SEQ ID NO:24	CP4 #5	(fragment) homolog to *C. pneumoniae* transglycolase/transpeptidase (pbp3 gene)
SEQ ID NO:25	CP4 #5	Polypeptide translation corresponding to SEQ ID NO. 24, homolog to *C. pneumoniae* transglycolase/transpeptidase(pbp3 gene)

SEQ ID NO:26	CP4 #5	(full length) homolog to *C. pneumoniae* transglycolase/transpeptidase (pbp3 gene)
SEQ ID NO:27	CP4 #5	Polypeptide translation corresponding to SEQ. ID NO. 26, homolog to *C. pneumoniae* transglycolase/transpeptidase (pbp3 gene)
SEQ ID NO:28	CP4 #6	(fragment) homolog to *C. pneumoniae* Protein Translocase (secA2 gene)
SEQ ID NO:29	CP4 #6	Polypeptide translation corresponding to SEQ ID NO. 28, homolog to *C. pneumoniae* Protein Translocase (secA2 gene)
SEQ ID NO:30	CP4 #13	(fragment) homolog to *C. pneumoniae* Protein Translocase (secA2 gene)
SEQ ID NO:31	CP4 #13	Polypeptide translation corresponding to SEQ ID NO. 30, homolog to *C. pneumoniae* Protein Translocase (secA2 gene)
SEQ ID NO:32	CP4 #6 & 13	(full length) homolog to *C. pneumoniae* Protein Translocase (secA2 gene)
SEQ ID NO:33	CP4 #6 & 13	Polypeptide translation corresponding to SEQ ID NO. 32, homolog to *C. pneumoniae* Protein Translocase (secA2 gene)
SEQ ID NO:34	CP4 #7	(fragment) homolog to *C. pneumoniae* Outer Membrane Lipoprotein (Cpn 0278)
SEQ ID NO:35	CP4 #7	Polypeptide translation corresponding to SEQ ID NO. 34, homolog to *C. pneumoniae* Outer Membrane Lipoprotein (Cpn 0278 gene)
SEQ ID NO:36	CP4 #7	(full length) homolog to *C. pneumoniae* Outer Membrane Lipoprotein (Cpn 0278)
SEQ ID NO:37	CP4 #7	Polypeptide translation corresponding to SEQ ID NO. 36, homolog to *C. pneumoniae* Outer Membrane Lipoprotein (Cpn 0278 gene)
SEQ ID NO:38	CP4 #8	(fragment) homolog to *C. pneumoniae* Oligopeptidase (pepF gene)

SEQ ID NO:39	CP4 #8	Polypeptide translation corresponding to SEQ ID NO. 38, homolog to *C. pneumoniae* Oligopeptidase (pepF gene)
SEQ ID NO:40	CP4 #8	(full length) homolog to *C. pneumoniae* Oligopeptidase (pepF gene)
SEQ ID NO:41	CP4 #8	Polypeptide translation corresponding to SEQ ID NO. 40, homolog to *C. pneumoniae* Oligopeptidase (pepF gene)
SEQ ID NO:42	CP4 #9	(fragment) homolog to *C. pneumoniae* gene of unknown function, co-translationaly coupled to Yop N Flagellar-Type ATPase (Cpn 0708 gene)
SEQ ID NO:43	CP4 #9	Polypeptide translation corresponding to SEQ ID NO. 42, homolog to *C. pneumoniae* gene of unknown function, co-translationally coupled to Yop N Flagellar-Type ATPase (Cpn 0708 gene)
SEQ ID NO:44	CP4 #9	(full length) homolog to *C. pneumoniae* gene of unknown function, co-translationally coupled to Yop N Flagellar-Type ATPase (Cpn 0708 gene)
SEQ ID NO:45	CP4 #9	Polypeptide translation corresponding to SEQ ID NO. 44, homolog to *C. pneumoniae* gene of unknown function, co-translationally coupled to Yop N Flagellar-Type ATPase (Cpn 0708 gene)
SEQ ID NO:46	CP4 #9	(full length) homolog to *C. pneumoniae* Yop N Flagellar-Type ATPase (yscN gene)
SEQ ID NO:47	CP4 #9	Polypeptide translation corresponding to SEQ ID NO. 46, homolog to *C. pneumoniae* Yop N Flagellar-Type ATPase (yscN gene)
SEQ ID NO:48	CP4 #10	(fragment) homolog to *C. pneumoniae* outer membrane protein G (pmp 2 gene) (Nucleotides 1-423 were previously sequenced by Longbottom *et al.*)
SEQ ID NO:49	CP4 #10	Polypeptide translation corresponding to SEQ ID NO. 48, homolog to *C. pneumoniae* outer membrane protein G (pmp 2 gene)

SEQ ID NO:50	CP4 #11	(fragment) homolog to *C. pneumoniae* outer membrane protein G (pmp 2 gene) (Nucleotides 1-301 were previously sequenced by Longbottom *et al.*)
SEQ ID NO:51	CP4 #11	Polypeptide translation corresponding to SEQ ID NO. 50, homolog to *C. pneumoniae* outer membrane protein G (pmp 2 gene)
SEQ ID NO:52	CP4 #10 & 11	(full length) homolog to *C. pneumoniae* outer membrane protein G (pmp 2 gene). This gene immediately follows the OMP90A gene on *C. psittaci*, and nucleotides 1-502 were published by Longbottom *et al.*, although they did not report this as a gene.
SEQ ID NO:53	CP4 #10 & 11	Polypeptide translation corresponding to SEQ ID NO. 52, homolog to *C. pneumoniae* outer membrane protein G (pmp 2 gene).
SEQ ID NO:54	CP4 #12	(fragment) *C. psittaci* 98 kDa outer membrane protein (POMP gene) (Previously sequenced by Longbottom, et al)
SEQ ID NO:55	CP4 #12	Polypeptide translation corresponding to SEQ ID NO. 54, *C. psittaci* 98 kDa outer membrane protein (POMP gene)
SEQ ID NO:56	CP4 #12	(full length) *C. psittaci* 98 kDa outer membrane protein (POMP gene) (Previously sequenced by Longbottom *et al.*)
SEQ ID NO:57	CP4 #12	Polypeptide translation corresponding to SEQ ID NO. 56, *C. psittaci* 98 kDa outer membrane protein (POMP gene)
SEQ ID NO:58	CP4 #14	(fragment) homolog to *C. pneumoniae* Succinate Dehydrogenase (sdhC)
SEQ ID NO:59	CP4 #14	Polypeptide translation corresponding to SEQ ID NO. 58, homolog to *C. pneumoniae* Succinate Dehydrogenase (sdhC gene)
SEQ ID NO:60	CP4 #14	(full length) homolog to *C. pneumoniae* Succinate Dehydrogenase (sdhC)

SEQ ID NO:61	CP4 #14	Polypeptide translation corresponding to SEQ ID NO. 60, homolog to *C. pneumoniae* Succinate Dehydrogenase (sdhC gene)

Of the 14 single genes identified in this study, ten are internal fragments and three contain the C-terminus of the protein. Of the five most protective clones (CP4 #1-5), one was from a putative outer membrane protein (CP4 #4) and one was from a cell surface protein (CP4 #5). The other three were from cytosolic proteins, with CP4 #2 and CP4 #3 deriving independently from genes encoding a particular amidotransferase complex.

Four of the 14 clones have sequence similarity to a class of proteins known as putative outer membrane proteins (POMPs) in *Chlamydia psittaci* and *Chlamydia pneumoniae* (CP4 #4, CP4 #10, CP4 #11 and CP4 #12). Many of the "putative" outer membrane proteins are known to be localized to the outer membrane and to be highly immunogenic (Longbottom et al., 1996; Tan et al., 1990). The clone designated CP4 #4 is an in-frame fragment of POMP90A (Longbottom et al., 1998) and CP4 #12 is an in-frame fragment of a 98 kDa POMP which has been completely sequenced (Accession U72499). The clones CP4 #10 and CP4 #11 immediately follow CP4 #4 in the genome and have sequence similarity to POMPs in *C. psittaci, C. trachomatis* and *C. pneumoniae*. As stated earlier, the clone CP4 #10 overlaps the CP4 #11 clone. Of these clones only CP4 #4 confers significant protection in isolation so clearly the criteria of being an outer membrane protein is not sufficient to predict a protective vaccine.

Example 7

Mixing Experiment

The two dimensional approach used to find protective gene fragments assumes that the protection is due to a single highly protective gene within a pool. To verify that such genes would be found, 25 ng (i.e. 1/2000) of either CP4 #4 or CP4 #11 was added to a pool that scored negative (pool 6 round 1). As depicted in FIG. 7, spiking with either clone converted the negative library to a positive. Of note is that CP4 #11 did not confer protection when tested individually, however, it does protect in combination.

The fact that a CP4 #4 positive library confers protection validates the sensitivity of the system. The fact that a CP4 #11 positive library protects implies that CP4 #11 can be a useful component of a vaccine, but that it may depend upon having other antigens present. A likely explaination is that CP4 #11 is a good vaccine antigen, but requires immunological help.

Example 8

Vaccination in Cattle

An important question is whether the genes identified in this manner in a mouse model are clinically relevant. Of course, this concern is not peculiar to genetic vaccines or ELI, but any system that uses models to identify vaccine candidates. In this case the clinically relevant situation is protection of cattle. In a preliminary experiment, the inventors evaluated the pool of 14 individual clones in the original host in a fertility challenge model. All fourteen clones were used as the individual test data on each clone in mice was not available by the time it was necessary to initiate the cow trial.

TABLE 4.

***C. psittaci* Vaccine in Cows**

	Percent Pregnant	Pregnant	Not Pregnant
Not Challenged	75	3	1
Challenged, Not Vaccinated	0	0	4
EB Vaccine	25	1	3
Genetic Vaccine (14 gene pool)	33	2	4

C. psittaci is normally introduced by the fecal-oral and respiratory routes in cattle, and disseminates to other tissues including reproductive organs. *C. psittaci* infection of

the uterine mucosa reduces fertility, the basis of the economic interest in a *C. psittaci* vaccine. Four groups of heifers were used. One group was the naïve unchallenged control, another the naïve, challenged control, a third received the same pool of fourteen gene fragments that were tested in mice, and the fourth group was vaccinated with an experimental, inactivated vaccine of elementary bodies (EB) and also challenged. This EB vaccine had shown great promise in field trials but is too expensive to produce. After a prime and one boost, the heifers were estrus synchronized by prostaglandin injection, were in heat 2-3 days later, and were artificially inseminated, simultaneously receiving an intracervical chlamydial challenge of $3x10^7$ inclusion forming units. The heifers were palpated for pregnancy at six weeks after insemination. This challenge was very high in order to maximize the difference between positive and negative control animals. This was necessary because only a small number of cows could be justified for this high-risk experiment.

Although the animal numbers are small, the results are quite encouraging. As is seen in Table 4, three out of four animals became pregnant in the positive control (non-challenged) group, 0/4 in the negative control (non-vaccinated, challenged) group, 2/6 in the genetic immunization group, and 1/4 in the elementary body vaccine group. The genetic vaccine of the pooled genes performed at least as well as the EB vaccine. Also relative to the inventor's interest in therapeutic vaccines, these cows were not sterile with respect to *C. psittaci* at the time of the prime inoculation. The vaccination was in the face of previous exposure and low level *C. psittaci* infection; as determined by the high titers of preinoculation antichlamydial antibodies, and occasional positivity of *Chlamydia ompl* PCRs from vaginal scrapings.

The next phase in developing a cow vaccine will be to experimentally verify the effectiveness of particular groups of the protective genes and then convert the codon usage of the *C. psittaci* genes to that of a mammal. This should increase the expression of the antigen in cows and increase the effectiveness of the vaccine. The inventors will test different combinations of those genes which have been found to be individually

protective, as well as combinations with CP4 #11. Both the original fragments and their full-length versions can be tested, both as nucleic acid segments and proteins. Once the combinations have been verified in mice or other small mammals, those combinations showing the most promise will be tested in cows. After immunization, the cows will be challenged with *C. psittaci*, either by direct challenge at insemination or infection by herd-mates. Direct challenge at insemination is a very severe and unnatural form of challenge. Therefore, even if protection is not demonstrated in the wake of such challenge, this does not necessarily mean that no protection has been conferred upon the cows.

All of the compositions and methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the compositions and methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

Example 9

Fertility at 42 days post breeding in heifers vaccinated with the pool of the 5 best mouse-protective genes of *Chlamydia psittaci*.

Because it is known that bacterial genes are not expressed efficiently in mammalian cells, the five most protective genes were chemically resynthesized to give an optimal mammalian codon bias. In addition, the full-length genes corresponding to the fragments isolated in the screen were used.

One group of five heifers was vaccinated with this pool. Another group of six heifers was vaccinated with an Alum-Quil A based vaccine containing per dose 100 μg each of the affinity-purified protein fragments expressed in *E. coli* from these genes. The control group of twelve heifers was vaccinated with a plasmid expressing an unrelated bacterial gene. Six weeks after the initial immunization all groups received booster vaccinations. Eight weeks later all heifers, including a cohort of 27 non-vaccinated heifers, were estrus-synchronized by prostaglandin injection. After coming into heat two to three days later, the non-vaccinated cohort heifers were infected with an intrauterine chlamydial inoculum of 10^8 IFUs *C. psittaci* B577. The function of this group was to shed chlamydiae, and thus to challenge through natural infection routes the vaccinated animals at the time of breeding. Eleven days later, the vaccinated animals were re-synchronized, and inseminated at estrus. The heifers were rectally palpated for pregnancy determination at six weeks after insemination.

The Genetic Vaccine group was vaccinated with DNA comprised of the pool of 5 full length, mammalianized genes, the Protein Vaccine group with the 5 full-length proteins, and the control group with DNA of an unrelated gene from *Salmonella typhimurium*. During the 3-week period prior to *C. psittaci* infection, heifers of all groups, including the non-vaccinated challenge cohort, shed low levels of *C. psittaci* (0.5±0.2 genomes/swab) as determined by qPCR of weekly collected vaginal cytobrush swabs. To challenge the vaccinated animals via natural transmission at the time of breeding, a cohort of 27 non-vaccinated animals was intracervically infected with *C. psittaci*. Eleven days later, all vaccinated groups were estrus-synchronized and inseminated. During the 4 weeks following the infection, the infected cohort animals shed high levels of chlamydiae (3826±2052 genomes per swab), and then returned to low baseline shedding (24.2±10.9 genomes per swab) for the remaining 5-week observation period. All vaccinated heifers were exposed to the natural challenge infection, as evident in their 7-fold increased post-breeding shedding of chlamydiae (3.6±1.2 genomes/swab; $p < 0.05$) compared to pre-breeding shedding of all heifers. No difference in chlamydial shedding before or after breeding was found between the *C. psittaci* vaccinated and the control vaccinated groups.

TABLE 5. Fertility in cows vaccinated with a pool of the 5 best mouse-protective *Chlamydia psittaci* genes.

Group	Percent Pregnant	Pregnant	Not Pregnant
Control Group	50	6	6
Genetic Vaccine	80	4	1
Protein Vaccine	83	5	1

As is seen in Table 5, six out of twelve animals (50 % fertility) became pregnant in the control group, 4/5 or (80 % fertility in the genetic vaccine group, and five out of six (83% fertility) in the protein vaccine group. Thus, 9/11 animals in both vaccine groups were pregnant. The genetic vaccine of the pooled genes performed as well as the protein vaccine.These fertility data correspond very well with typical data of bovine herds with and without fertility problems. When both vaccine groups combined are compared to the controls, the 1-tailed Fisher's exact test indicates with a $p = 0.122$ that vaccination is effective to improve *Chlamydia*-induced reduction of fertility. The odds ratio for improvement of fertility by vaccination is 4.5 (0.67-30.23, 95% confidence interval). These data are important in view of the fact that all heifers in the experiment had been previously exposed to chlamydiae and experienced low-level herd infection with *C. psittaci*, as determined by positive *C. psittaci* B577 MOMP-peptide ELISA and sporadic detection by quantitative PCR of low levels of *C. psittaci* in pre-challenge vaginal cytobrush swabs.

REFERENCES

The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference.

U. S. Patent No. 5,670,488

U. S. Patent No. 5,770,414

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U.S. Patent No. 5,858,744

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WHAT IS CLAIMED IS:

1. An isolated polynucleotide comprising a region having a sequence of SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:58, or SEQ ID NO:60, or fragment thereof.

2. An isolated polynucleotide comprising a region having a sequence having at least 17 contiguous nucleotides in common with at least one of SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:58, or SEQ ID NO:60, or its complement.

3. The isolated polynucleotide of claim 2, further defined as comprising a sequence having least 50 contiguous nucleotides in common with at least one of SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:58, or SEQ ID NO:60, or its complement.

4. The isolated polynucleotide of claim 3, further defined as comprising a sequence having all nucleotides in common with at least one of SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:58, or SEQ ID NO:60, or its complement.

5. A polypeptide having a sequence of SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:59, SEQ ID NO:61 or fragment thereof.

6. The polypeptide of claim 5, further defined as a recombinant polypeptide.

7. A method of producing a polypeptide having a sequence of SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:59, SEQ ID NO:61 or fragment thereof, comprising:

a) obtaining a polynucleotide comprising a region encoding the sequence; and

b) expressing the polynucleotide to obtain the polypeptide.

8. The method of claim 7, wherein the polynucleotide has a region having a sequence of SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:58, or SEQ ID NO:60, or fragment thereof

9. An antibody directed against an antigen having sequence of SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID

NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, or SEQ ID NO:61, or antigenic fragment thereof.

10. The antibody of claim 9, further defined as a monoclonal antibody.

11. A vaccine for the immunization of an animal against *Chlamydia psittaci* comprising:

(a) a pharmaceutically acceptable carrier, and

(b) at least one polynucleotide having a *Chlamydia psittaci* sequence.

12. The vaccine of claim 11, further defined as a vaccine for the immunization of a bovine.

13. The vaccine of claim 11, wherein the at least one polynucleotide has a sequence isolated from a *Chlamydia psittaci* genomic DNA expression library

14. The vaccine of claim 11, wherein the at least one polynucleotide has a sequence of SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO52:, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO: 58, or SEQ ID NO:60, or fragment thereof.

15. The vaccine of claim 14, wherein the at least one polynucleotide has a sequence of SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, or SEQ ID NO:26, or fragment thereof.

16. The vaccine of claim 14, wherein the at least one polynucleotide has a sequence of SEQ ID NO:6, SEQ ID NO:10, SEQ ID NO:14, SEQ ID NO:20, or SEQ ID NO:24.

17. The vaccine of claim 11, wherein the polynucleotide is comprised in a genetic immunization vector.

18. The vaccine of claim 17, wherein the vector comprises a gene encoding a mouse ubiquitin fusion polypeptide.

19. The vaccine of claim 17, wherein the vector comprises a promoter operable in eukaryotic cells.

20. The vaccine of claim 19, wherein the promoter is a CMV promoter.

21. The vaccine of claim 11, wherein the polynucleotide is cloned into a viral expression vector.

22. The vaccine of claim 21, wherein the viral expression vector is selected from the group consisting of adenovirus, adeno-associated virus, retrovirus and herpes-simplex virus.

23. The vaccine of claim 11, comprising a polynucleotide encoding aN antigen having a sequence of SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, or SEQ ID NO:61, or antigenic fragment thereof.

24. The vaccine of claim 11, comprising at least a first polynucleotide having a *Chlamydia psittaci* sequence and a second polynucleotide having a *Chlamydia psittaci*

sequence, wherein the first polynucleotide and the second polynucleotide have different *Chlamydia psittaci* sequences.

25. The vaccine of claim 24, wherein the first polynucleotide has a sequence of SEQ ID NO:50.

26. A vaccine for the immunization of an animal against *Chlamydia psittaci* comprising:

(a) a pharmaceutically acceptable carrier; and

(b) at least one *Chlamydia psittaci* antigen.

27. The vaccine of claim 26, further defined as a vaccine for the immunization of a bovine.

28. The vaccine of claim 26, wherein the at least one *Chlamydia psittaci* antigen has a sequence of SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, or SEQ ID NO:61, or antigenic fragment thereof.

29. The vaccine of claim 26, wherein the at least one *Chlamydia psittaci* antigen has a sequence of SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, or SEQ ID NO:27, or an antigenic fragment thereof.

30. The vaccine of claim 26, wherein the at least one *Chlamydia psittaci* antigen has a sequence of SEQ ID NO:7, SEQ ID NO:11, SEQ ID NO:15, SEQ ID NO:21, or SEQ ID NO:25.

31. A method of immunizing a bovine comprising providing to the bovine at least one *Chlamydia psittaci* antigen, or antigenic fragment thereof, in an amount effective to induce an immune response.

32. The method of claim 31, wherein the at least one *Chlamydia psittaci* antigen has a sequence of SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, or SEQ ID NO:61, or an antigenic fragment thereof.

33. The method of claim 31, wherein the provision of the at least one *Chlamydia psittaci* antigen comprises:

(a) preparing a cloned expression library from fragmented genomic DNA, cDNA or sequenced genes of *Chlamydia psittaci*;

(b) administering at least one clone of the library in a pharmaceutically acceptable carrier into the bovine; and

(c) expressing at least one *Chlamydia psittaci* antigen in the bovine.

34. The method of claim 33, wherein the expression library comprises at least one or more polynucleotide having a sequence of SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO52:, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO: 58, or SEQ ID NO:60, or fragment thereof.

35. The method of claim 34, wherein the expression library is cloned in a genetic immunization vector of SEQ ID NO:1.

36. The method of claim 35, wherein the vector comprises a gene encoding a mouse ubiquitin fusion polypeptide designed to link the expression library polynucleotides to the ubiquitin gene.

37. The method of claim 36, wherein the vector comprises a promoter operable in eukaryotic cells.

38. The method of claim 37, wherein the promoter is a CMV promoter.

39. The method of claim 34, wherein the polynucleotide is administered by a intramuscular injection or epidermal injection.

40. The method of claim 34, wherein the polynucleotide is administered by intravenous, subcutaneous, intralesional, intraperitoneal, oral or inhaled routes of administration.

41. The method of claim 39, wherein a second intramuscular injection and epidermal injection are administered at least about three weeks after the first injection.

42. The method of claim 34, wherein the polynucleotide is cloned into a viral expression vector.

43. The method of claim 31, wherein the provision of the *Chlamydia psittaci* antigen(s) comprises:

(a) preparing a pharmaceutical composition comprising at least one polynucleotide having a sequence of SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24,

SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO52:, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO: 58, or SEQ ID NO:60, or fragment thereof;

(b) administering one or more clones of the library in a pharmaceutically acceptable carrier into the bovine; and

(c) expressing one or more *Chlamydia psittaci* antigens in the bovine.

44. The method of claim 43, wherein the one or more polynucleotides is in one or more expression vectors.

45. The method of claim 44, wherein the one or more polynucleotides is cloned in a genetic immunization vector of SEQ ID NO:1.

46. The method of claim 31, wherein the provision of the *Chlamydia* antigen(s) comprises:

(a) preparing a pharmaceutical composition of at least one *Chlamydia* antigen having a sequence of SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SFQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, SEQ ID NO:57, SEQ ID NO:59, or SEQ ID NO:61, or an antigenic fragment thereof; and

(b) administering the at least one antigen or fragment into the bovine.

47. The method of claim 46, wherein the antigen(s) is administered by a first intramuscular injection, intravenous injection, parenteral injection, epidermal injection, inhalation or oral route.

48. The method of claim 47, wherein a second intramuscular injection, intravenous injection, parenteral injection or epidermal injection is administered at least about three weeks after the first injection.

49. A method of assaying for the presence of *Chlamydia psittaci* infection in an animal comprising:

(a) obtaining an antibody directed against a *Chlamydia psittaci* antigen;

(b) obtaining a sample from the animal;

(c) admixing the antibody with the sample; and

(d) assaying the sample for antigen-antibody binding,

wherein the antigen-antibody binding indicates *Chlamydia psittaci* infection in the animal.

50. The method of claim 49, wherein the animal is a bovine.

51. The method of claim 49, wherein the antibody directed against the antigen is further defined as a monoclonal antibody.

52. The method of claim 49, wherein assaying the sample for antigen-antibody binding is by precipitin reaction, radioimmunoassay, ELISA, Western blot or immunofluorescence.

53. A kit for assaying a *Chlamydia psittaci* infection comprising, in a suitable container:

(a) a pharmaceutically acceptable carrier; and

(b) an antibody directed against a *Chlamydia psittaci* antigen.

54. A method of assaying for the presence of a *Chlamydia psittaci* infection in a bovine comprising:

(a) obtaining an oligonucleotide probe comprising a sequence comprised within SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO52:, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO: 58, or SEQ ID NO:60, or a complement thereof; and

(b) employing the probe in a PCR detection protocol.

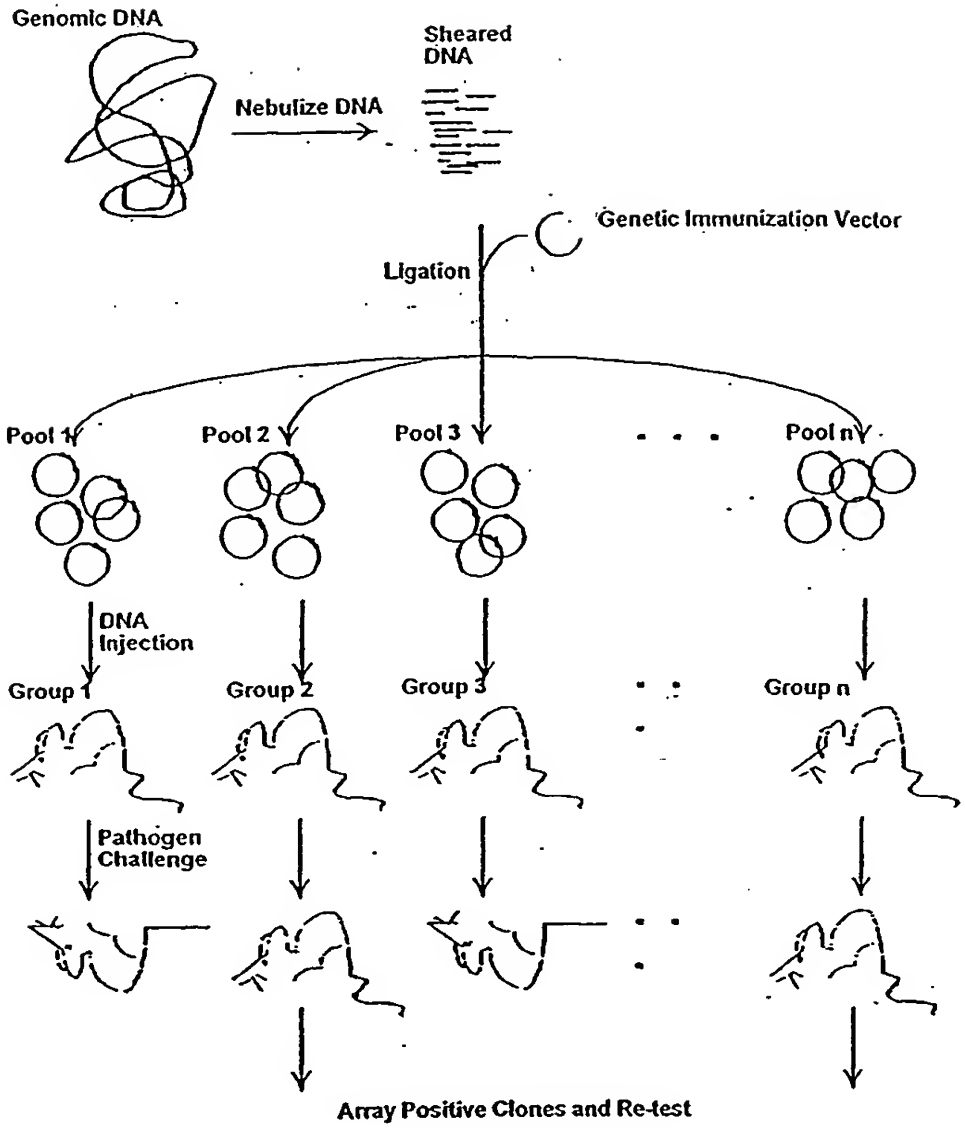
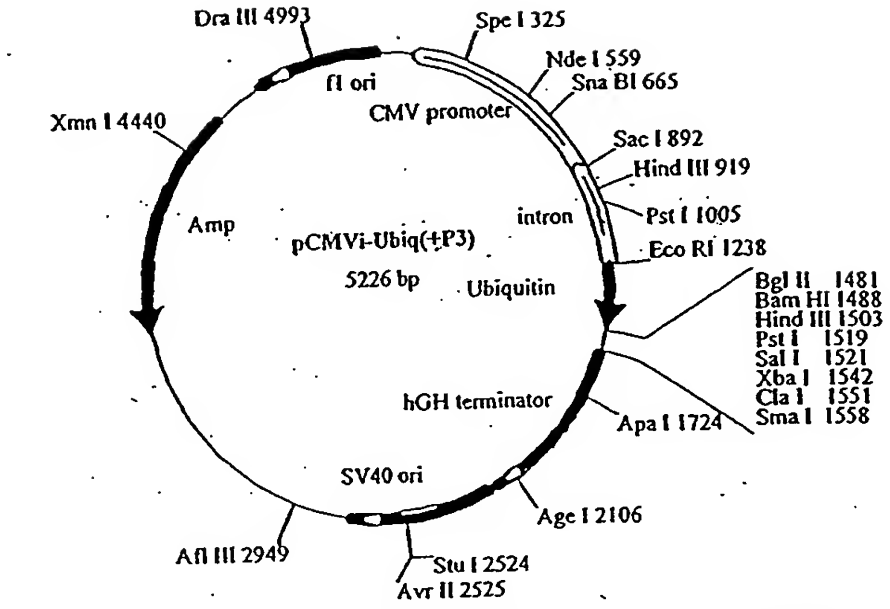

Genomic DNA
Sheared DNA
Nebulize DNA
Genetic Immunization Vector
Ligation
Pool 1
Pool 2
Pool 3
Pool n
DNA Injection
Group 1
Group 2
Group 3
Group n
Pathogen Challenge
Array Positive Clones and Re-test

FIG. 1



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Ubiquitin
Bgl II
Bam HI

stop stop stop stop
AGT AAG CTT GCA TGC CTG CAG GTC GAC TCT AGG TGA CTA ATA TCT AGA
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Pst I
Sal I
Xba I

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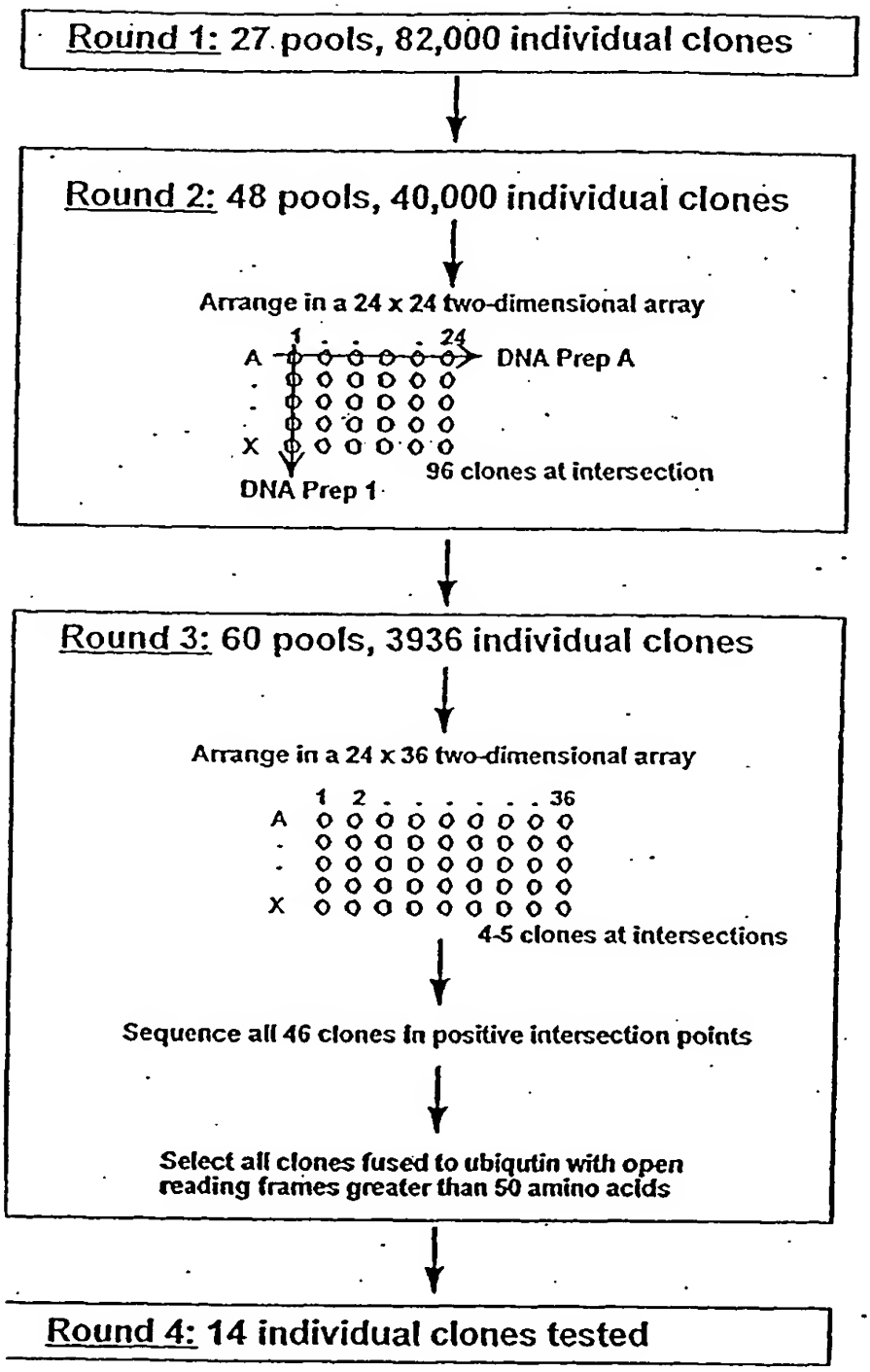
Cla I
Sma I

FIG. 2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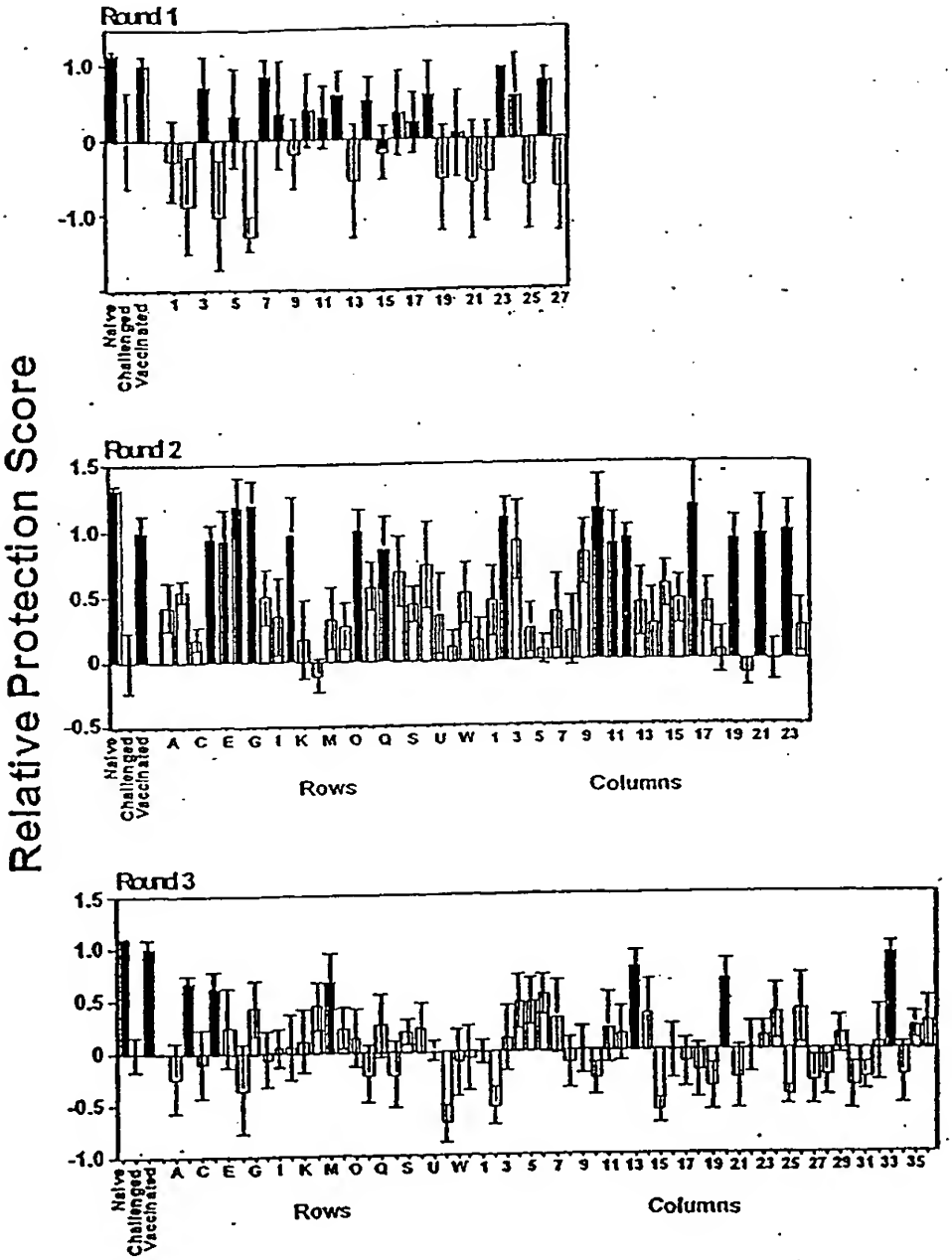
Round 2: 48 pools, 40,000 individual clones
Arrange in a 24 x 24 two-dimensional array
1
24
A
DNA Prep A
X
DNA Prep 1
96 clones at intersection
Round 3: 60 pools, 3936 individual clones
Arrange in a 24 x 36 two-dimensional array
1 2 36
A
X
4-5 clones at intersections
Sequence all 46 clones in positive intersection points
Select all clones fused to ubiqutin with open reading frames greater than 50 amino acids
Round 4: 14 individual clones tested

FIG. 3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0
-1.0
1 3 5 7 9 11 13 15 17 19 21 23 25 27
Naïve
Challenged
Vaccinated
Round 2
1.5
1.0
0.5
0
-0.5
A C E G I K M O Q S U W 1 3 5 7 9 11 13 15 17 19 21 23
Naïve
Challenged
Vaccinated
Rows
Columns
Relative Protection Score
Round 3
1.5
1.0
0.5
0
-0.5
-1.0
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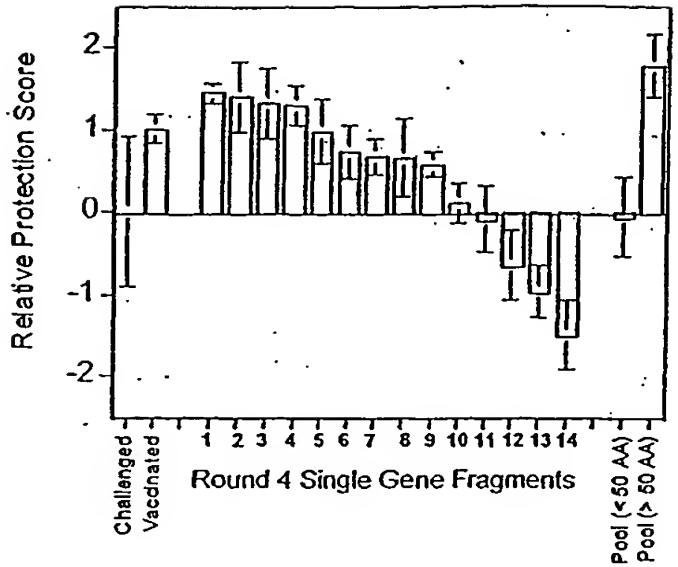
Naïve
Challenged
Vaccinated
Rows
Columns

FIG. 4


Relative Protection Score
2
1
0
-1
-2
Challenged
Vaccinated
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Round 4 Single Gene Fragments
Pool (< 50 AA)
Pool (> 50 AA)

FIG. 5

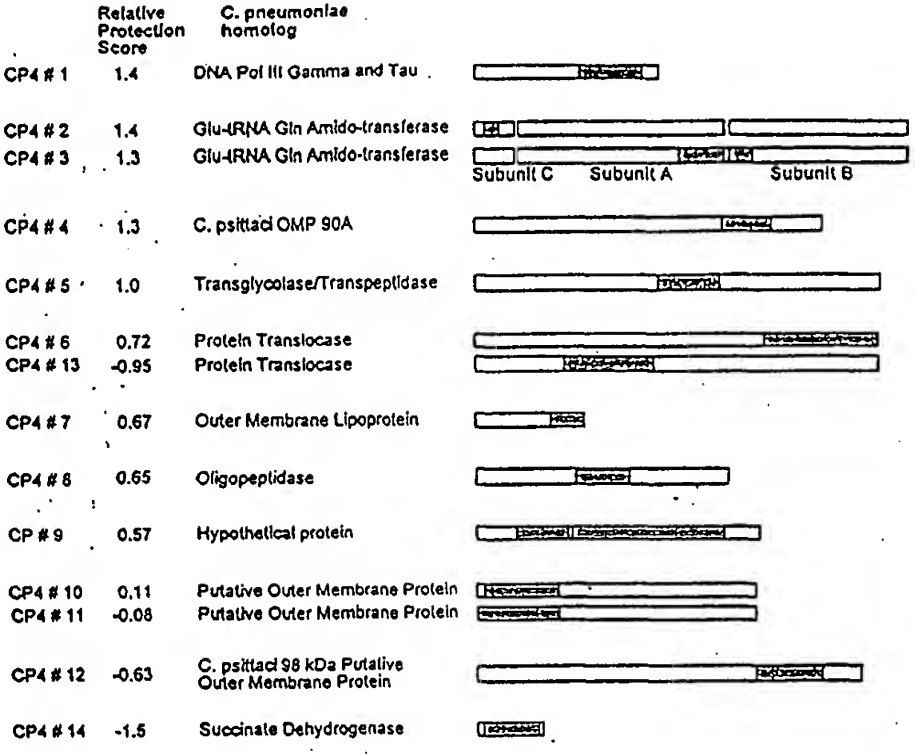

FIG. 6

Chlamydia psittaci Addition Experiments

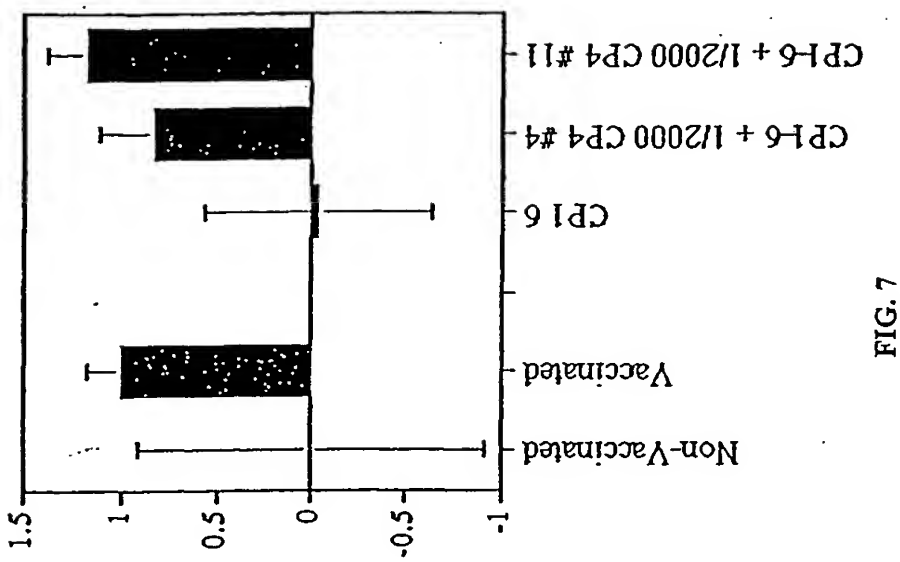

FIG. 7

SEQUENCE LISTING

<110> JOHNSTON, STEPHEN A.
STEMKE-HALE, KATHERINE
SYKES, KATHRYN F.
KALTENBOECK, BERNHARD

<120> METHODS AND COMPOSITIONS FOR VACCINATION COMPRISING NUCLEIC ACID AND/OR POLYPEPTIDE SEQUENCES OF CHLAMYDIA PSITTACI

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     50                  55                  60

Ile Ile Asp Phe Leu Gly Glu Ala Ala Lys His Leu Gln Gln Thr Ile
 65                  70                  75                  80

Phe Glu Lys Thr Phe Leu Glu Thr Val Ile Ile His Leu Ile Arg Ile
                 85                  90                  95

Cys Gln Arg Pro Ser Leu Glu Thr Leu Phe Ser Gln Leu Lys Thr Ser
            100                 105                 110

Thr Phe Asp Thr Val Arg Asn Val Pro Gln Gln Gln Glu Pro Ser Lys
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        35                  40                  45

Ile Arg Gly Thr Gly Lys Thr Thr Leu Ala Arg Ile Phe Ala Lys Ala
    50                  55                  60

Leu Asn Cys Lys Glu Leu Thr Pro Glu His Glu Pro Cys Asn Gln Cys
65                  70                  75                  80

Cys Val Cys Lys Glu Ile Ser Ser Gly Thr Ser Leu Asp Val Ile Glu
                85                  90                  95

Ile Asp Gly Ala Ser His Arg Gly Ile Glu Asp Ile Arg Gln Ile Asn
            100                 105                 110

Glu Thr Val Leu Phe Thr Pro Ala Lys Ser Gln Tyr Lys Ile Tyr Ile
        115                 120                 125

Ile Asp Glu Val His Met Leu Thr Lys Glu Ala Phe Asn Ser Leu Leu
    130                 135                 140

Lys Thr Leu Glu Glu Pro Pro Ser His Val Lys Phe Phe Leu Ala Thr
145                 150                 155                 160

Thr Glu Asn Tyr Lys Ile Pro Ser Thr Ile Leu Ser Arg Cys Gln Lys
                165                 170                 175

Met His Leu Lys Arg Ile Pro Glu Thr Met Ile Val Asp Lys Leu Ala
            180                 185                 190

Ser Ile Ser Gln Ala Gly Gly Ile Glu Thr Ser Arg Glu Ala Leu Leu
        195                 200                 205

Pro Ile Ala Arg Ala Ala Gln Gly Ser Leu Arg Asp Ala Glu Ser Leu
    210                 215                 220

Tyr Asp Tyr Val Ile Gly Leu Phe Pro Thr Ser Leu Ser Pro Glu Leu
225                 230                 235                 240

Val Ala Asp Ala Leu Gly Leu Leu Ser Gln Asp Thr Leu Ala Thr Leu
                245                 250                 255

Ser Glu Cys Ile Arg Thr Gln Lys Tyr Ala Glu Ala Leu Leu Pro Val
            260                 265                 270
```

```
Thr Thr Ala Ile Asn Ser Gly Val Ala Pro Ile Thr Phe Leu His Asp
        275                 280                 285

Leu Thr Val Phe Tyr Arg Asp Val Leu Leu Asn Lys Asp Gln Gly Asn
    290                 295                 300

Ser Pro Leu Ser Ala Ile Ala Met His Tyr Ser Ser Glu Cys Leu Leu
305                 310                 315                 320

Glu Ile Ile Asp Phe Leu Gly Glu Ala Ala Lys His Leu Gln Gln Thr
                325                 330                 335

Ile Phe Glu Lys Thr Phe Leu Glu Thr Val Ile Ile His Leu Ile Arg
            340                 345                 350

Ile Cys Gln Arg Pro Ser Leu Glu Thr Leu Phe Ser Gln Leu Lys Thr
        355                 360                 365

Ser Thr Phe Asp Thr Val Arg Asn Val Pro Gln Gln Gln Glu Pro Ser
    370                 375                 380

Lys Pro Ser Ile Gln Pro Glu Lys His Tyr Gln Asp Gln Ser Phe Leu
385                 390                 395                 400

Thr Ser Pro Ser Pro Thr Pro Lys Val Gln His Gln Lys Glu Ala Ser
                405                 410                 415

Pro Ser Leu Val Gly Ser Ala Thr Ile Asp Thr Leu Leu Gln Phe Ala
            420                 425                 430

Val Val Glu Phe Ser Gly Ile Leu Thr Lys Glu
        435                 440
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<210> 10
<211> 123
<212> DNA
<213> Chlamydia psittaci

<400> 10
gagtttattc aagagtatga aagttcttta aatgaagtca ttaaaactat ggcagcatcc 60
atcgctatgg atgtaaccga cgtggttatt gaggttggtt tatcccatgt gatcagtccc 120
gaa 123

<210> 11
<211> 41
<212> PRT
<213> Chlamydia psittaci

<400> 11

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Glu Phe Ile Gln Glu Tyr Glu Ser Ser Leu Asn Glu Val Ile Lys Thr
  1               5                  10                  15

Met Ala Ala Ser Ile Ala Met Asp Val Thr Asp Val Val Ile Glu Val
             20                  25                  30
```

Gly Leu Ser His Val Ile Ser Pro Glu
35 40

<210> 12
<211> 303
<212> DNA
<213> Chlamydia psittaci

<400> 12
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gaattaagcg aagagtttat tcaagagtat gaaagttctt taaatgaagt cattaaaact 120
atggcagcat ccatcgctat ggatgtaacc gacgtggtta ttgaggttgg tttatcccat 180
gtgatcagtc ccgaagattt acgagaagat atcgttgcct caagtttctc tcgtgaggag 240
tttctaacta atgtccctga atccttaggg ggattagtaa aagtacccac agtcattaag 300
tag 303

<210> 13
<211> 100
<212> PRT
<213> Chlamydia psittaci

<400> 13
Met Thr Gln Pro Tyr Val Thr Arg Glu Asp Ile Ile Leu Leu Ala Lys
1 5 10 15

Ser Ser Ala Leu Glu Leu Ser Glu Glu Phe Ile Gln Glu Tyr Glu Ser
20 25 30

Ser Leu Asn Glu Val Ile Lys Thr Met Ala Ala Ser Ile Ala Met Asp
35 40 45

Val Thr Asp Val Val Ile Glu Val Gly Leu Ser His Val Ile Ser Pro
50 55 60

Glu Asp Leu Arg Glu Asp Ile Val Ala Ser Ser Phe Ser Arg Glu Glu
65 70 75 80

Phe Leu Thr Asn Val Pro Glu Ser Leu Gly Gly Leu Val Lys Val Pro
85 90 95

Thr Val Ile Lys
100

<210> 14
<211> 514
<212> DNA
<213> Chlamydia psittaci

<400> 14
gaaaagtgtg atgtgattgc gatgcctgta tgctcatgcc cagcattcgc cgatggcgaa 60
atccttgatc ctacctctct atatctccag gatatctata ccgtggctat gaatttagcc 120
tacctcccag ctatcgccgt tccttcaggg ttttctcgag aagggctgcc tctaggattc 180
caggtgattg gacaaaaggg taaagatcaa caggtgtgcc aggtaggcta tagcttccaa 240

gaacattcag gaattaagaa tttatacccct aaaggatgta acaaacttgt tgatggagag 300
gtgaaataat gagcgacgtt tatgctgatt gggaatccgt cataggtctt gaagtccacg 360
tagaattaaa cacaaaatct aaattgttca gttgtgcacg caaccgtttt ggagacgaac 420
ctaatacaaa catctctcct gtatgcaccg gcatgccggg gtcactgcca gtactgaata 480
aagaagcagt gagaaaggct gttttatttg gttg 514

<210> 15
<211> 102
<212> PRT
<213> Chlamydia psittaci

<400> 15
Glu Lys Cys Asp Val Ile Ala Met Pro Val Cys Ser Cys Pro Ala Phe
1 5 10 15

Ala Asp Gly Glu Ile Leu Asp Pro Thr Ser Leu Tyr Leu Gln Asp Ile
20 25 30

Tyr Thr Val Ala Met Asn Leu Ala Tyr Leu Pro Ala Ile Ala Val Pro
35 40 45

Ser Gly Phe Ser Arg Glu Gly Leu Pro Leu Gly Phe Gln Val Ile Gly
50 55 60

Gln Lys Gly Lys Asp Gln Gln Val Cys Gln Val Gly Tyr Ser Phe Gln
65 70 75 80

Glu His Ser Gly Ile Lys Asn Leu Tyr Pro Lys Gly Cys Asn Lys Leu
85 90 95

Val Asp Gly Glu Val Lys
100

<210> 16
<211> 1476
<212> DNA
<213> Chlamydia psittaci

<400> 16
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acagcaatag caaagtattt ttataataga ataaaaacag aagacaatca gataggagct 120
tttctttctc tttgtgaaga aagagcttat gagaaagcag ctatcataga tgcgaaagtg 180
gcgcgaggag aacctttggg gaaactcgca ggtgtcccca tcgggataaa agataatatt 240
catattcggg gtttgcgcac cacttgtgct tctaaaatgt tagaaaatta tatagcgcct 300
tttgatgcta cagtcgtcga acggatagaa gctgaagatg gggtcatttt aggcaaactc 360
aatatggatg agtttgctat gggatcgaca acgcagtatt ctgctttcca tcctacgaaa 420
aatccttggg gtttatcctg tgtgccagga ggatcttcag ggggatccgc cgccgcagtt 480
tctgcaagat tttgtcctat agcgttaggt tcggataccg gtggatctat acgtcagcca 540
gcagcatttt gtggagttgt ggggtttaag ccctcctatg gagccgtctc ccgttacggt 600
ttagtcgctt ttgggtcttc attagatcag ataggccctt taacaacagt tgtcgaagat 660
gtcgccttag ctatggatgt attcgcaggt aaggatgata gagatgcaac ttctcagaag 720
ttttttacag gatctttcca agaggccttg tctttagacg ttccgagttt gatcggcgtg 780
cctatgggat ttttagacgg tttacgtgat gatgttaaag agaatttctt tgcctcttta 840
agtattttgg aacgtcaggg tagccgcatt gttgaagtgg atcttaacat cttagatcac 900
gctgtctctg tttactacat tgtcgcttct gcagaagccg caacaaatct tgcaagattt 960

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atctcccgcg tacaaggctt cggtaaggaa gtcatgcgta ggattctttt aggtaactat 1080
gtgttatcca ctgagcgcca aaatgtctat tataagaaag gctccgcaat tcgagcaaaa 1140
atcattcaag cttttcaaaa agcttatgaa aagtgtgatg tgattgcgat gcctgtatgc 1200
tcatgcccag cattcgccga tggcgaaatc cttgatccta cctctctata tctccaggat 1260
atctataccg tggctatgaa tttagcctac ctcccagcta tcgccgttcc ttcagggttt 1320
tctcgagaag ggctgcctct aggattccag gtgattggac aaaagggtaa agatcaacag 1380
gtgtgccagg taggctatag cttccaagaa cattcaggaa ttaagaattt ataccctaaa 1440
ggatgtaaca aacttgttga tggagaggtg aaataa                           1476
```

<210> 17
<211> 491
<212> PRT
<213> Chlamydia psittaci

<400> 17

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Met Tyr Gln Lys Ser Ala Leu Glu Leu Arg Asn Ala Val Val Ser Gly
  1               5                  10                  15

Glu Ser Ser Ala Thr Ala Ile Ala Lys Tyr Phe Tyr Asn Arg Ile Lys
            20                  25                  30

Thr Glu Asp Asn Gln Ile Gly Ala Phe Leu Ser Leu Cys Glu Glu Arg
        35                  40                  45

Ala Tyr Glu Lys Ala Ala Ile Ile Asp Ala Lys Val Ala Arg Gly Glu
    50                  55                  60

Pro Leu Gly Lys Leu Ala Gly Val Pro Ile Gly Ile Lys Asp Asn Ile
65                  70                  75                  80

His Ile Arg Gly Leu Arg Thr Thr Cys Ala Ser Lys Met Leu Glu Asn
                85                  90                  95

Tyr Ile Ala Pro Phe Asp Ala Thr Val Val Glu Arg Ile Glu Ala Glu
            100                 105                 110

Asp Gly Val Ile Leu Gly Lys Leu Asn Met Asp Glu Phe Ala Met Gly
        115                 120                 125

Ser Thr Thr Gln Tyr Ser Ala Phe His Pro Thr Lys Asn Pro Trp Gly
    130                 135                 140

Leu Ser Cys Val Pro Gly Gly Ser Ser Gly Gly Ser Ala Ala Ala Val
145                 150                 155                 160

Ser Ala Arg Phe Cys Pro Ile Ala Leu Gly Ser Asp Thr Gly Gly Ser
                165                 170                 175

Ile Arg Gln Pro Ala Ala Phe Cys Gly Val Val Gly Phe Lys Pro Ser
            180                 185                 190

Tyr Gly Ala Val Ser Arg Tyr Gly Leu Val Ala Phe Gly Ser Ser Leu
        195                 200                 205

Asp Gln Ile Gly Pro Leu Thr Thr Val Val Glu Asp Val Ala Leu Ala
```

210 215 220

Met Asp Val Phe Ala Gly Lys Asp Asp Arg Asp Ala Thr Ser Gln Lys
225 230 235 240

Phe Phe Thr Gly Ser Phe Gln Glu Ala Leu Ser Leu Asp Val Pro Ser
245 250 255

Leu Ile Gly Val Pro Met Gly Phe Leu Asp Gly Leu Arg Asp Asp Val
260 265 270

Lys Glu Asn Phe Phe Ala Ser Leu Ser Ile Leu Glu Arg Gln Gly Ser
275 280 285

Arg Ile Val Glu Val Asp Leu Asn Ile Leu Asp His Ala Val Ser Val
290 295 300

Tyr Tyr Ile Val Ala Ser Ala Glu Ala Ala Thr Asn Leu Ala Arg Phe
305 310 315 320

Asp Gly Ile Arg Tyr Gly Tyr Arg Ser Pro Glu Ala His Ser Ile Glu
325 330 335

Asp Ile Tyr Thr Ile Ser Arg Val Gln Gly Phe Gly Lys Glu Val Met
340 345 350

Arg Arg Ile Leu Leu Gly Asn Tyr Val Leu Ser Thr Glu Arg Gln Asn
355 360 365

Val Tyr Tyr Lys Lys Gly Ser Ala Ile Arg Ala Lys Ile Ile Gln Ala
370 375 380

Phe Gln Lys Ala Tyr Glu Lys Cys Asp Val Ile Ala Met Pro Val Cys
385 390 395 400

Ser Cys Pro Ala Phe Ala Asp Gly Glu Ile Leu Asp Pro Thr Ser Leu
405 410 415

Tyr Leu Gln Asp Ile Tyr Thr Val Ala Met Asn Leu Ala Tyr Leu Pro
420 425 430

Ala Ile Ala Val Pro Ser Gly Phe Ser Arg Glu Gly Leu Pro Leu Gly
435 440 445

Phe Gln Val Ile Gly Gln Lys Gly Lys Asp Gln Gln Val Cys Gln Val
450 455 460

Gly Tyr Ser Phe Gln Glu His Ser Gly Ile Lys Asn Leu Tyr Pro Lys
465 470 475 480

Gly Cys Asn Lys Leu Val Asp Gly Glu Val Lys
485 490

<210> 18
<211> 1464
<212> DNA

<213> Chlamydia psittaci

<400> 18
atgagcgacg tttatgctga ttgggaatcc gtcataggtc ttgaagtcca cgtagaatta 60
aacacaaaat ctaaattgtt cagttgtgca cgcaaccgtt ttggagacga acctaataca 120
aacatctctc ctgtatgcac cggcatgccg gggtcactgc cagtactgaa taaagaagca 180
gtgagaaagg ctgttttatt tggttgtgct gttgaaggcg aagtagcttt gctcagccgt 240
tttgatagaa agtcctattt ttatcccgat agcccaagga attttcaaat tacccaattc 300
gaacatccta ttgtgcgagg aggacatata aaagctatcg ttcacggtga ggaacgtcat 360
tttgaactgg ctcaagcgca tatcgaagat gatgccggta tgctaaaaca tttcggagaa 420
tttgctggag tagattataa ccgcgctggt gtacctttaa tagagattgt gtctaagccg 480
tgcatgtttt gtgctgatga tgctgttgct tatgccacag ctttggtatc cttattagac 540
tacataggca tttctgactg taatatggaa gaaggctcgg tacgctttga tgtaaacata 600
tccgtacgtc ctaaaggtag cgaagaacta cgcaataaag tagaaattaa aaatatgaac 660
tcctttgctt ttatggccca agctctagaa gccgagcgtt gtcgtcagat cgatgcatat 720
ttagacaatc caaatgcaga ccccaaaact gttattccag gagcgacata ccgttgggat 780
cctgaaaaga aaaaaacagt gttgatgcgt cttaaggaac gagctgaaga ttacaagtat 840
ttcatagagc ctgatctccc agtattgcaa ttaacagaag catatattga tgaaattcgt 900
catacgcttc ccgagctccc tttcaacaaa taccaaaggt atttgcacga atatgctctt 960
gccgaagaca tcgctgccat tttaattagc gataagcata gtgcgcactt ctttgaatta 1020
gccgctcagg aatgtaaaaa ctacagagcc ctttctaatt ggttaactgt tgagtttgcc 1080
ggacgttgta aactcaaggg taagaatctc gctttctcag gtatcctgcc cagtagtgta 1140
gctcagcttg tgaattttat tgatcaaggc gtgattaccg gaaagatcgc taaggatatc 1200
gcagacatga tgatggaatc tcctgaaaag agtcctgaga ctatcctcaa agaaaatcct 1260
gaaatgttgc ccatgacaga tgaaagtgcg ttggtggcga tcatttccga ggtgattacc 1320
gcaaatccgc agtctgtcgt agactacaaa agtggtaaga ccaaggcgtt aggattttta 1380
gttgggcaaa ttatgaaacg tacccagggc aaggcccctc caaatagggt aaatgaactt 1440
ttgcttgtgg aattaagtaa ataa 1464

<210> 19
<211> 487
<212> PRT
<213> Chlamydia psittaci

<400> 19
Met Ser Asp Val Tyr Ala Asp Trp Glu Ser Val Ile Gly Leu Glu Val
1 5 10 15

His Val Glu Leu Asn Thr Lys Ser Lys Leu Phe Ser Cys Ala Arg Asn
20 25 30

Arg Phe Gly Asp Glu Pro Asn Thr Asn Ile Ser Pro Val Cys Thr Gly
35 40 45

Met Pro Gly Ser Leu Pro Val Leu Asn Lys Glu Ala Val Arg Lys Ala
50 55 60

Val Leu Phe Gly Cys Ala Val Glu Gly Glu Val Ala Leu Leu Ser Arg
65 70 75 80

Phe Asp Arg Lys Ser Tyr Phe Tyr Pro Asp Ser Pro Arg Asn Phe Gln
85 90 95

Ile Thr Gln Phe Glu His Pro Ile Val Arg Gly Gly His Ile Lys Ala
100 105 110

```
Ile Val His Gly Glu Glu Arg His Phe Glu Leu Ala Gln Ala His Ile
        115                 120                 125

Glu Asp Asp Ala Gly Met Leu Lys His Phe Gly Glu Phe Ala Gly Val
    130                 135                 140

Asp Tyr Asn Arg Ala Gly Val Pro Leu Ile Glu Ile Val Ser Lys Pro
145                 150                 155                 160

Cys Met Phe Cys Ala Asp Asp Ala Val Ala Tyr Ala Thr Ala Leu Val
                165                 170                 175

Ser Leu Leu Asp Tyr Ile Gly Ile Ser Asp Cys Asn Met Glu Glu Gly
            180                 185                 190

Ser Val Arg Phe Asp Val Asn Ile Ser Val Arg Pro Lys Gly Ser Glu
        195                 200                 205

Glu Leu Arg Asn Lys Val Glu Ile Lys Asn Met Asn Ser Phe Ala Phe
    210                 215                 220

Met Ala Gln Ala Leu Glu Ala Glu Arg Cys Arg Gln Ile Asp Ala Tyr
225                 230                 235                 240

Leu Asp Asn Pro Asn Ala Asp Pro Lys Thr Val Ile Pro Gly Ala Thr
                245                 250                 255

Tyr Arg Trp Asp Pro Glu Lys Lys Lys Thr Val Leu Met Arg Leu Lys
            260                 265                 270

Glu Arg Ala Glu Asp Tyr Lys Tyr Phe Ile Glu Pro Asp Leu Pro Val
        275                 280                 285

Leu Gln Leu Thr Glu Ala Tyr Ile Asp Glu Ile Arg His Thr Leu Pro
    290                 295                 300

Glu Leu Pro Phe Asn Lys Tyr Gln Arg Tyr Leu His Glu Tyr Ala Leu
305                 310                 315                 320

Ala Glu Asp Ile Ala Ala Ile Leu Ile Ser Asp Lys His Ser Ala His
                325                 330                 335

Phe Phe Glu Leu Ala Ala Gln Glu Cys Lys Asn Tyr Arg Ala Leu Ser
            340                 345                 350

Asn Trp Leu Thr Val Glu Phe Ala Gly Arg Cys Lys Leu Lys Gly Lys
        355                 360                 365

Asn Leu Ala Phe Ser Gly Ile Leu Pro Ser Ser Val Ala Gln Leu Val
    370                 375                 380

Asn Phe Ile Asp Gln Gly Val Ile Thr Gly Lys Ile Ala Lys Asp Ile
385                 390                 395                 400

Ala Asp Met Met Met Glu Ser Pro Glu Lys Ser Pro Glu Thr Ile Leu
                405                 410                 415
```

```
Lys Glu Asn Pro Glu Met Leu Pro Met Thr Asp Glu Ser Ala Leu Val
            420                 425                 430

Ala Ile Ile Ser Glu Val Ile Thr Ala Asn Pro Gln Ser Val Val Asp
        435                 440                 445

Tyr Lys Ser Gly Lys Thr Lys Ala Leu Gly Phe Leu Val Gly Gln Ile
    450                 455                 460

Met Lys Arg Thr Gln Gly Lys Ala Pro Pro Asn Arg Val Asn Glu Leu
465                 470                 475                 480

Leu Leu Val Glu Leu Ser Lys
                485
```

<210> 20
<211> 379
<212> DNA
<213> Chlamydia psittaci

<400> 20
tatttagtgt cgaaaaacaa cgccaacatt tacgcaggtt ctctctatta tcagcatatc 60
tcctattgga gcgcttggca gaatctgcta caaaacacta tcggtgcaga agctccgtta 120
gtccttaacg cacagttaac ttattgtcat gcttcaaacg acatgaaaac caacatgacg 180
actacttacg ctcctcgtaa aacaacgtat gcagaaatca agggtgattg gggtaacgat 240
tgtttcggag tcgagcttgg tgcaactgtg cctatccaaa cagaatcttc tctcctattc 300
gatatgtact caccttttcct gaagtttcaa cttgtgcata cgcaccaaga tgactttaag 360
gaaaacaata gcgatcagg 379

<210> 21
<211> 126
<212> PRT
<213> Chlamydia psittaci

<400> 21
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Tyr Leu Val Ser Lys Asn Asn Ala Asn Ile Tyr Ala Gly Ser Leu Tyr
  1               5                  10                  15

Tyr Gln His Ile Ser Tyr Trp Ser Ala Trp Gln Asn Leu Leu Gln Asn
            20                  25                  30

Thr Ile Gly Ala Glu Ala Pro Leu Val Leu Asn Ala Gln Leu Thr Tyr
        35                  40                  45

Cys His Ala Ser Asn Asp Met Lys Thr Asn Met Thr Thr Thr Tyr Ala
    50                  55                  60

Pro Arg Lys Thr Thr Tyr Ala Glu Ile Lys Gly Asp Trp Gly Asn Asp
65                  70                  75                  80

Cys Phe Gly Val Glu Leu Gly Ala Thr Val Pro Ile Gln Thr Glu Ser
                85                  90                  95

Ser Leu Leu Phe Asp Met Tyr Ser Pro Phe Leu Lys Phe Gln Leu Val
            100                 105                 110
```

His Thr His Gln Asp Asp Phe Lys Glu Asn Asn Ser Asp Gln
115 120 125

<210> 22
<211> 2520
<212> DNA
<213> Chlamydia psittaci

<400> 22
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agcttcgcta acgacgctca aacagcctta actccctccg atagctataa tggaaatgtg 120
acctctgagg agttccaggt aaaagaaact tcatcaggaa caacgtatac ttgtgaaggc 180
aatgtgtgta tctcctttgc agggaaagat tcaggtctaa agaaaagttg tttctcagct 240
actgataacc ttaccttcct aggaaacggg tatactcttt gctttgataa tattactact 300
acagctagta accccggagc cattaatgtt caaggtcaag gaaaaacctt aggcatctca 360
ggattttctt tattttcatg tgcttattgt cctccaggca caactggtta cggagctata 420
cagactaaag gcaacacaac tttaaaagat aactctagtc ttgtcttcca taaaaactgc 480
tcaacagcag aaggtggggc tatccagtgt aaaggaagca gtgatgctga attaaaaata 540
gaaaataatc agaatctggt tttctcagaa aactcctcca cttcaaaagg cggggctatt 600
tatgctgata aactcaccat tgtctcaggt gggcctacat tattttctaa caactctgta 660
tccaacggtt catcccctaa aggcggagct attagcataa aagattcaag tggtgaatgt 720
agcctaaccg ctgatctcgg agatattacc ttcgatggga acaaaatcat caaaactagt 780
ggtggaagtt ctacagtaac aagaaattcc atagatctcg gcacagggaa atttacaaag 840
ctacgtgcta aagacggctt cggaattttc ttctatgacc ctattactgg gggaggatct 900
gatgaactaa acattaataa aaaagaaact gttgattata caggaaagat cgtcttctca 960
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aaacaagtaa cgcaggaagc gggatctacc gttgtcatgg atctagggac cacattacag 1140
acgccttctt caggtggaga aaccatcacc ctaactaatc tagatattaa catcgcctcg 1200
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ataactatta acgctgtcaa tctagtcgat gctgatggca atgcttatga agatcctatt 1320
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taccatagag gtttttgggt atccggtcta gctaacttct tacacaaaag tggctctgat 1680
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ttagtgtcga aaaacaacgc caacatttac gcaggttctc tctattatca gcatatctcc 1860
tattggagcg cttggcagaa tctgctacaa aacactatcg gtgcagaagc tccgttagtc 1920
cttaacgcac agttaactta ttgtcatgct tcaaacgaca tgaaaaccaa catgacgact 1980
acttacgctc ctcgtaaaac aacgtatgca gaaatcaagg gtgattgggg taacgattgt 2040
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atgtactcac ctttcctgaa gtttcaactt gtgcatacgc accaagatga ctttaaggaa 2160
aacaatagcg atcagggaag atacttcgaa agcagcaatc tcaccaacct ttctctgcct 2220
atcggcatca agtttgagag atttgctaac aacgatacag cttcttatca tgtcactgct 2280
gcttattctc ctgatatcgt aagaagtaac cctgactgta ctacttctct gttagtaagc 2340
cccgactctg ctgtctgggt aacgaaagcc aacaaccttg cgcgaagcgc cttcatgcta 2400
caagcaggaa actacttgtc tttaagtcac aacatagaaa tcttcagcca gttcggtttc 2460
gagctcaggg gatcttcacg aacctataac gtagatctcg gatcgaagat ccagttctaa 2520

<210> 23
<211> 839

<212> PRT
<213> Chlamydia psittaci

<400> 23

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Met Lys His Pro Val Tyr Trp Phe Leu Ile Ser Ser Ser Leu Phe Ala
  1               5                  10                  15

Ser Asn Ser Leu Ser Phe Ala Asn Asp Ala Gln Thr Ala Leu Thr Pro
             20                  25                  30

Ser Asp Ser Tyr Asn Gly Asn Val Thr Ser Glu Glu Phe Gln Val Lys
         35                  40                  45

Glu Thr Ser Ser Gly Thr Thr Tyr Thr Cys Glu Gly Asn Val Cys Ile
     50                  55                  60

Ser Phe Ala Gly Lys Asp Ser Gly Leu Lys Lys Ser Cys Phe Ser Ala
 65                  70                  75                  80

Thr Asp Asn Leu Thr Phe Leu Gly Asn Gly Tyr Thr Leu Cys Phe Asp
                 85                  90                  95

Asn Ile Thr Thr Thr Ala Ser Asn Pro Gly Ala Ile Asn Val Gln Gly
            100                 105                 110

Gln Gly Lys Thr Leu Gly Ile Ser Gly Phe Ser Leu Phe Ser Cys Ala
        115                 120                 125

Tyr Cys Pro Pro Gly Thr Thr Gly Tyr Gly Ala Ile Gln Thr Lys Gly
    130                 135                 140

Asn Thr Thr Leu Lys Asp Asn Ser Ser Leu Val Phe His Lys Asn Cys
145                 150                 155                 160

Ser Thr Ala Glu Gly Gly Ala Ile Gln Cys Lys Gly Ser Ser Asp Ala
                165                 170                 175

Glu Leu Lys Ile Glu Asn Asn Gln Asn Leu Val Phe Ser Glu Asn Ser
            180                 185                 190

Ser Thr Ser Lys Gly Gly Ala Ile Tyr Ala Asp Lys Leu Thr Ile Val
        195                 200                 205

Ser Gly Gly Pro Thr Leu Phe Ser Asn Asn Ser Val Ser Asn Gly Ser
    210                 215                 220

Ser Pro Lys Gly Gly Ala Ile Ser Ile Lys Asp Ser Ser Gly Glu Cys
225                 230                 235                 240

Ser Leu Thr Ala Asp Leu Gly Asp Ile Thr Phe Asp Gly Asn Lys Ile
                245                 250                 255

Ile Lys Thr Ser Gly Gly Ser Ser Thr Val Thr Arg Asn Ser Ile Asp
            260                 265                 270

Leu Gly Thr Gly Lys Phe Thr Lys Leu Arg Ala Lys Asp Gly Phe Gly
        275                 280                 285
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Ile Phe Phe Tyr Asp Pro Ile Thr Gly Gly Gly Ser Asp Glu Leu Asn
    290                 295                 300

Ile Asn Lys Lys Glu Thr Val Asp Tyr Thr Gly Lys Ile Val Phe Ser
305                 310                 315                 320

Gly Glu Lys Leu Ser Asp Glu Glu Lys Ala Arg Ala Glu Asn Leu Ala
                325                 330                 335

Ser Thr Phe Asn Gln Pro Ile Thr Leu Ser Ala Gly Ser Leu Val Leu
            340                 345                 350

Lys Asp Gly Val Ser Val Thr Ala Lys Gln Val Thr Gln Glu Ala Gly
        355                 360                 365

Ser Thr Val Val Met Asp Leu Gly Thr Thr Leu Gln Thr Pro Ser Ser
    370                 375                 380

Gly Gly Glu Thr Ile Thr Leu Thr Asn Leu Asp Ile Asn Ile Ala Ser
385                 390                 395                 400

Leu Gly Gly Gly Gly Gly Thr Ser Pro Ala Lys Leu Ala Thr Asn Thr
                405                 410                 415

Ala Ser Gln Ala Ile Thr Ile Asn Ala Val Asn Leu Val Asp Ala Asp
            420                 425                 430

Gly Asn Ala Tyr Glu Asp Pro Ile Leu Ala Thr Ser Lys Pro Phe Thr
        435                 440                 445

Ala Ile Val Ala Thr Thr Asn Ala Ser Thr Val Thr Gln Pro Thr Asp
    450                 455                 460

Asn Leu Thr Asn Tyr Val Pro Pro Thr His Tyr Gly Tyr Gln Gly Asn
465                 470                 475                 480

Trp Thr Val Thr Trp Asp Thr Glu Thr Ala Thr Lys Thr Ala Thr Leu
                485                 490                 495

Thr Trp Glu Gln Thr Gly Tyr Ser Pro Asn Pro Glu Arg Gln Gly Pro
            500                 505                 510

Leu Val Pro Asn Thr Leu Trp Gly Ala Phe Ser Asp Leu Arg Ala Ile
        515                 520                 525

Gln Asn Leu Met Asp Ile Ser Val Asn Gly Ala Asp Tyr His Arg Gly
    530                 535                 540

Phe Trp Val Ser Gly Leu Ala Asn Phe Leu His Lys Ser Gly Ser Asp
545                 550                 555                 560

Thr Lys Arg Lys Phe Arg His Asn Ser Ala Gly Tyr Ala Leu Gly Val
                565                 570                 575

Tyr Ala Lys Thr Pro Ser Asp Asp Ile Phe Ser Ala Ala Phe Cys Gln
            580                 585                 590
```

```
Leu Phe Gly Lys Asp Lys Asp Tyr Leu Val Ser Lys Asn Asn Ala Asn
        595                 600                 605

Ile Tyr Ala Gly Ser Leu Tyr Tyr Gln His Ile Ser Tyr Trp Ser Ala
    610                 615                 620

Trp Gln Asn Leu Leu Gln Asn Thr Ile Gly Ala Glu Ala Pro Leu Val
625                 630                 635                 640

Leu Asn Ala Gln Leu Thr Tyr Cys His Ala Ser Asn Asp Met Lys Thr
                645                 650                 655

Asn Met Thr Thr Thr Tyr Ala Pro Arg Lys Thr Thr Tyr Ala Glu Ile
            660                 665                 670

Lys Gly Asp Trp Gly Asn Asp Cys Phe Gly Val Glu Leu Gly Ala Thr
        675                 680                 685

Val Pro Ile Gln Thr Glu Ser Ser Leu Leu Phe Asp Met Tyr Ser Pro
    690                 695                 700

Phe Leu Lys Phe Gln Leu Val His Thr His Gln Asp Asp Phe Lys Glu
705                 710                 715                 720

Asn Asn Ser Asp Gln Gly Arg Tyr Phe Glu Ser Ser Asn Leu Thr Asn
                725                 730                 735

Leu Ser Leu Pro Ile Gly Ile Lys Phe Glu Arg Phe Ala Asn Asn Asp
            740                 745                 750

Thr Ala Ser Tyr His Val Thr Ala Ala Tyr Ser Pro Asp Ile Val Arg
        755                 760                 765

Ser Asn Pro Asp Cys Thr Thr Ser Leu Leu Val Ser Pro Asp Ser Ala
    770                 775                 780

Val Trp Val Thr Lys Ala Asn Asn Leu Ala Arg Ser Ala Phe Met Leu
785                 790                 795                 800

Gln Ala Gly Asn Tyr Leu Ser Leu Ser His Asn Ile Glu Ile Phe Ser
                805                 810                 815

Gln Phe Gly Phe Glu Leu Arg Gly Ser Ser Arg Thr Tyr Asn Val Asp
            820                 825                 830

Leu Gly Ser Lys Ile Gln Phe
        835
```

<210> 24
<211> 1039
<212> DNA
<213> Chlamydia psittaci

<400> 24
aaacgttttc atattaatgg ggttcctgaa tggtctttat ctacgcctta ttctcttgct 60

atggggtata atatcttggc tacgggagtg cagatggtta aagcctatgc cattcttgcc 120
aacggtggtt atgatgtgcg ccctaccttg ataaaaaaaa tagtcactac ttctggaaaa 180
gagtacgtgt tgcatcctca agttcgtgga gaaagaattc tttctcagga cattgtggat 240
gaggtattga aagctacgcg ttttactacc tatcctggag gaacgggatt tcgggctgcg 300
cctaaaaagc attccagtgc agggaaaaca ggaacaacag aaaagctagt tcatggaaag 360
tatgataagc atcggcatat ttcttcattt ataggtatca cgccgatata cccttcggca 420
gggggggagtg ttcctttggt catgcttgtc tctatcagtt atacgaccga caacggtagt 480
caagtgtacg tcgttcaatt gcgacatgag ggtatcgaaa tctgtcgtca attcgtccat 540
gttaacctaa ttgtgtggtc attatcgctt tctttatact acttaccgta gttcctacgg 600
atactagcaa aaagttctgc tctttgcgtt gctctttgaa cagcatactg tacttttaaa 660
aagtctgcta aattttcccg ttctccattc ctatctgaga agtagagaag ggctctattt 720
aacacttctt ctccagaaga cacccaattg accatcttac gggcaacgga ctcgtgttct 780
tcttcttttt tggtttgtaa gttttgttgc gtatgctcag ctatatcatt cagatcacca 840
ttgattaaat caatgatcac actgacagct tcaaaatgtt cttgcgatag tttattttga 900
tcttgttgta gagtggattg tgcatcccat aaacgctctt ttagattgtt tatttgctct 960
ttcagctctt ccgaatctaa cgcctcttcc agttcaggat cgataatgtt agagtttctg 1020
tcttgcatca tcgccatag 1039

<210> 25
<211> 196
<212> PRT
<213> Chlamydia psittaci

<400> 25
Lys Arg Phe His Ile Asn Gly Val Pro Glu Trp Ser Leu Ser Thr Pro
1 5 10 15

Tyr Ser Leu Ala Met Gly Tyr Asn Ile Leu Ala Thr Gly Val Gln Met
20 25 30

Val Lys Ala Tyr Ala Ile Leu Ala Asn Gly Gly Tyr Asp Val Arg Pro
35 40 45

Thr Leu Ile Lys Lys Ile Val Thr Thr Ser Gly Lys Glu Tyr Val Leu
50 55 60

His Pro Gln Val Arg Gly Glu Arg Ile Leu Ser Gln Asp Ile Val Asp
65 70 75 80

Glu Val Leu Lys Ala Thr Arg Phe Thr Thr Tyr Pro Gly Gly Thr Gly
85 90 95

Phe Arg Ala Ala Pro Lys Lys His Ser Ser Ala Gly Lys Thr Gly Thr
100 105 110

Thr Glu Lys Leu Val His Gly Lys Tyr Asp Lys His Arg His Ile Ser
115 120 125

Ser Phe Ile Gly Ile Thr Pro Ile Tyr Pro Ser Ala Gly Gly Ser Val
130 135 140

Pro Leu Val Met Leu Val Ser Ile Ser Tyr Thr Thr Asp Asn Gly Ser
145 150 155 160

Gln Val Tyr Val Val Gln Leu Arg His Glu Gly Ile Glu Ile Cys Arg
165 170 175

Gln Phe Val His Val Asn Leu Ile Val Trp Ser Leu Ser Leu Ser Leu
180 185 190

Tyr Tyr Leu Pro
195

<210> 26
<211> 1950
<212> DNA
<213> Chlamydia psittaci

<400> 26
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ttaggacaac atgaatttcg agtaaaggac ccttttcgta gggggacgtt tttttctcag 180
atgaatttac gtaagggaga ttcagagcaa cgacaagctc tggccgtgga cattacgaaa 240
tttcatcttt gtttagatgc tgtagctgtt cctgaagaac accgtgatgt gattgctaag 300
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aaatctcgct atcgaaagtt atttctttgg ttagatcgtg cggatcatga ccgcatcctg 420
tcttggtggc gggggtacgc agcaaaatct aaaataccct cgaatgcttt gtttttcatg 480
accgactatc aaagatctta tccctttggc aaacttttag gccaagttct acatactctg 540
agagaagtca aggatgagaa aacaggcaaa gctttcccta caggaggttt agaagcctat 600
tttaaccacg tccttgaagg agagccagga gaacggaaat tcctacgttc tcctttaaat 660
cgtttagatc tagataaagt cacaaagatt cctagggatg gttcggatat ttatctcaca 720
gtcaatccct gtatacagac tatagcggaa gaggaattag aaaaaggggt aaaggaagcc 780
aaagctaaag gtgggcgtct aattttaatg aatgcttata caggcgagat tcttgcttta 840
gcacagtatc ctttctttaa tccttcggaa tacaaggaat ttttcaatga taaggaaaaa 900
atagagcaca caaaagtaac atcagtcagt gatgtgtttg aacccggctc tatcatgaaa 960
cctctgactc tggctatagc gttgctggcc aacgaagaga tggtgaaaag atcaggaaag 1020
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caatttccgc ttaaggatat ctcatcgaat cggcgtttaa atatgtacat ggcgattcaa 1140
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cactggtatg aagacaagtt attgttatta ggatttggta aaaagacggg gatagaattg 1260
ccaggggaag cgtcaggatt ggtaccttca cctaaacgtt ttcatattaa tggggttcct 1320
gaatggtctt tatctacgcc ttattctctt gctatggggt ataatatctt ggctacggga 1380
gtgcagatgg ttaaagccta tgccattctt gccaacggtg gttatgatgt gcgccctacc 1440
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acaggaacaa cagaaaagct agttcatgga aagtatgata agcatcggca tatttcttca 1680
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gtctctatag atgatcctga tcattgtgtt cgcgaggatg gaacaaagaa ctatatggga 1800
ggccgatgtg ccgcccctgt atttggcaga gttgcggatc gtgttttatc ttatctagga 1860
gttcccgaag ataaagaaaa atacagttat cagagtgagg tggctgctat gaaagctttg 1920
tatgaggaat ggaatcgttc ggggaaataa 1950

<210> 27
<211> 649
<212> PRT
<213> Chlamydia psittaci

<400> 27
Met Asn His Arg Lys Cys Leu Thr Met Ile Thr Tyr Gly Val Leu Leu
1 5 10 15

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Ser Tyr Ser Phe Leu Ile Ile Arg Tyr Tyr Lys Ile Gln Ile Cys Glu
            20                  25                  30

Glu Lys Arg Trp Ala Ala Glu Ala Leu Gly Gln His Glu Phe Arg Val
        35                  40                  45

Lys Asp Pro Phe Arg Arg Gly Thr Phe Phe Ser Gln Met Asn Leu Arg
    50                  55                  60

Lys Gly Asp Ser Glu Gln Arg Gln Ala Leu Ala Val Asp Ile Thr Lys
65                  70                  75                  80

Phe His Leu Cys Leu Asp Ala Val Ala Val Pro Glu Glu His Arg Asp
                85                  90                  95

Val Ile Ala Lys Lys Val Phe Ser Leu Ile Gly Glu Gly Asp Tyr Asp
            100                 105                 110

Lys Leu Arg Ala Glu Phe Asp Lys Lys Ser Arg Tyr Arg Lys Leu Phe
        115                 120                 125

Leu Trp Leu Asp Arg Ala Asp His Asp Arg Ile Leu Ser Trp Trp Arg
    130                 135                 140

Gly Tyr Ala Ala Lys Ser Lys Ile Pro Ser Asn Ala Leu Phe Phe Met
145                 150                 155                 160

Thr Asp Tyr Gln Arg Ser Tyr Pro Phe Gly Lys Leu Leu Gly Gln Val
                165                 170                 175

Leu His Thr Leu Arg Glu Val Lys Asp Glu Lys Thr Gly Lys Ala Phe
            180                 185                 190

Pro Thr Gly Gly Leu Glu Ala Tyr Phe Asn His Val Leu Glu Gly Glu
        195                 200                 205

Pro Gly Glu Arg Lys Phe Leu Arg Ser Pro Leu Asn Arg Leu Asp Leu
    210                 215                 220

Asp Lys Val Thr Lys Ile Pro Arg Asp Gly Ser Asp Ile Tyr Leu Thr
225                 230                 235                 240

Val Asn Pro Cys Ile Gln Thr Ile Ala Glu Glu Glu Leu Glu Lys Gly
                245                 250                 255

Val Lys Glu Ala Lys Ala Lys Gly Gly Arg Leu Ile Leu Met Asn Ala
            260                 265                 270

Tyr Thr Gly Glu Ile Leu Ala Leu Ala Gln Tyr Pro Phe Phe Asn Pro
        275                 280                 285

Ser Glu Tyr Lys Glu Phe Phe Asn Asp Lys Glu Lys Ile Glu His Thr
    290                 295                 300

Lys Val Thr Ser Val Ser Asp Val Phe Glu Pro Gly Ser Ile Met Lys
305                 310                 315                 320
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Pro Leu Thr Leu Ala Ile Ala Leu Leu Ala Asn Glu Glu Met Val Lys
                325                 330                 335

Arg Ser Gly Lys Pro Leu Phe Asp Pro Asn Glu Pro Ile Asp Val Thr
            340                 345                 350

Arg Arg Ile Phe Pro Gly Arg Lys Gln Phe Pro Leu Lys Asp Ile Ser
        355                 360                 365

Ser Asn Arg Arg Leu Asn Met Tyr Met Ala Ile Gln Lys Ser Ser Asn
    370                 375                 380

Val Tyr Val Ala Gln Leu Ala Asp Leu Ile Val Gln His Leu Gly Asn
385                 390                 395                 400

His Trp Tyr Glu Asp Lys Leu Leu Leu Leu Gly Phe Gly Lys Lys Thr
                405                 410                 415

Gly Ile Glu Leu Pro Gly Glu Ala Ser Gly Leu Val Pro Ser Pro Lys
            420                 425                 430

Arg Phe His Ile Asn Gly Val Pro Glu Trp Ser Leu Ser Thr Pro Tyr
        435                 440                 445

Ser Leu Ala Met Gly Tyr Asn Ile Leu Ala Thr Gly Val Gln Met Val
    450                 455                 460

Lys Ala Tyr Ala Ile Leu Ala Asn Gly Gly Tyr Asp Val Arg Pro Thr
465                 470                 475                 480

Leu Ile Lys Lys Ile Val Thr Thr Ser Gly Lys Glu Tyr Val Leu His
                485                 490                 495

Pro Gln Val Arg Gly Glu Arg Ile Leu Ser Gln Asp Ile Val Asp Glu
            500                 505                 510

Val Leu Lys Ala Thr Arg Phe Thr Thr Tyr Pro Gly Gly Thr Gly Phe
        515                 520                 525

Arg Ala Ala Pro Lys Lys His Ser Ser Ala Gly Lys Thr Gly Thr Thr
    530                 535                 540

Glu Lys Leu Val His Gly Lys Tyr Asp Lys His Arg His Ile Ser Ser
545                 550                 555                 560

Phe Ile Gly Ile Thr Pro Ile Tyr Pro Ser Ala Gly Gly Ser Val Pro
                565                 570                 575

Leu Val Met Leu Val Ser Ile Asp Asp Pro Asp His Cys Val Arg Glu
            580                 585                 590

Asp Gly Thr Lys Asn Tyr Met Gly Gly Arg Cys Ala Ala Pro Val Phe
        595                 600                 605

Gly Arg Val Ala Asp Arg Val Leu Ser Tyr Leu Gly Val Pro Glu Asp
    610                 615                 620
```

```
Lys Glu Lys Tyr Ser Tyr Gln Ser Glu Val Ala Ala Met Lys Ala Leu
625                 630                 635                 640

Tyr Glu Glu Trp Asn Arg Ser Gly Lys
                645
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<210> 28
<211> 960
<212> DNA
<213> Chlamydia psittaci

<400> 28
atgttcaata agctcattga aacagcacag aaacgggtgg aagcaagaaa ctatactatt 60
cgaaagcata ctcttgagta tgacgatgtt atgaataggc aaaggcagac gatctatgct 120
tttcgtaatg acgttatccg ctctgaagat atctttggtt tagctaagga agcaatatct 180
catgttgcat taatgatcgc ttcgttgata gtgagccgtg atcatcctac agggaattct 240
cttcctaggc tggaagaatg gatgaactat tctttcccac tgcaattgaa tattgaagaa 300
ttgaaaagat tgaagtctat agatgccatt gccgaacggg ttgctgatga tctcatagaa 360
gttttccaga ataagtttgc ttctatggtg caggaaatta ccgaagcagc cggagaaaaa 420
gtcgatgcta atggtgtctg taaagatgtt attcgctcgg tcatgattat gcatatcgat 480
gagcagtgga aaattcatct tgtagatatg gatttattac gtagtgaagt aggtttacgt 540
actgtcggtc agaaagaccc tcttatcgaa tttaaacatg agtcgttctt actattcgaa 600
agtcttattc gcgatattcg tattgctatt gtaaagcatt tgttccgttt agagttgacg 660
atgactagag aacagcggcc tcaaaatgtc gtgcctgttg ttgccacatc tttccaaaat 720
aatgaaaatt tcggtccttt ggaactcaca gttatcagtg attctgacga tgaataaaaa 780
gagctttagg gctgggctag cttccagcct tttcccttac gttattgatt tatagtttta 840
aataaatacg gaccactcag accaggattg tgtgtcgtgg tggcgtatcc aaaatgttct 900
gtgattatcc tcaatcagaa attgtacatg atgatcgcga ttgcgtgttg tcatgcaaat 960

<210> 29
<211> 258
<212> PRT
<213> Chlamydia psittaci

<400> 29
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Met Phe Asn Lys Leu Ile Glu Thr Ala Gln Lys Arg Val Glu Ala Arg
  1               5                  10                  15

Asn Tyr Thr Ile Arg Lys His Thr Leu Glu Tyr Asp Asp Val Met Asn
             20                  25                  30

Arg Gln Arg Gln Thr Ile Tyr Ala Phe Arg Asn Asp Val Ile Arg Ser
         35                  40                  45

Glu Asp Ile Phe Gly Leu Ala Lys Glu Ala Ile Ser His Val Ala Leu
     50                  55                  60

Met Ile Ala Ser Leu Ile Val Ser Arg Asp His Pro Thr Gly Asn Ser
 65                  70                  75                  80

Leu Pro Arg Leu Glu Glu Trp Met Asn Tyr Ser Phe Pro Leu Gln Leu
                 85                  90                  95

Asn Ile Glu Glu Leu Lys Arg Leu Lys Ser Ile Asp Ala Ile Ala Glu
```

100 105 110

Arg Val Ala Asp Asp Leu Ile Glu Val Phe Gln Asn Lys Phe Ala Ser
115 120 125

Met Val Gln Glu Ile Thr Glu Ala Ala Gly Glu Lys Val Asp Ala Asn
130 135 140

Gly Val Cys Lys Asp Val Ile Arg Ser Val Met Ile Met His Ile Asp
145 150 155 160

Glu Gln Trp Lys Ile His Leu Val Asp Met Asp Leu Leu Arg Ser Glu
165 170 175

Val Gly Leu Arg Thr Val Gly Gln Lys Asp Pro Leu Ile Glu Phe Lys
180 185 190

His Glu Ser Phe Leu Leu Phe Glu Ser Leu Ile Arg Asp Ile Arg Ile
195 200 205

Ala Ile Val Lys His Leu Phe Arg Leu Glu Leu Thr Met Thr Arg Glu
210 215 220

Gln Arg Pro Gln Asn Val Val Pro Val Val Ala Thr Ser Phe Gln Asn
225 230 235 240

Asn Glu Asn Phe Gly Pro Leu Glu Leu Thr Val Ile Ser Asp Ser Asp
245 250 255

Asp Glu

<210> 30
<211> 697
<212> DNA
<213> Chlamydia psittaci

<400> 30
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gggttttatt ttgctattat cgatgaagtc gactcgattt taattgatga agccagaact 120
cctttaatta tttctggtcc tggggaaaaa cataatcctg tgtatttcga actcaaagat 180
aaagtggctg acctcgttca gttacaaagg gagttatgta accagttagc tcttgaagct 240
agacggggac tagaattgtt cttggatatg gatattcttc ctaaggataa aaaagttatc 300
gaagctatct ccgaattttg ccgtagctta tggttagtta gtaagggaat gcctttaaat 360
cgtgttttgc gtagagtgcg cgaacaccca gatttgcgag ccatgataga taaatgggat 420
acttattatc atgctgagca aaataaagaa gagagtatag agaagctatc tcagctgtat 480
atcattgttg atgaacataa taacgatttt gaattgacag atcgtggcat gcaacaatgg 540
gtggataagg ctggaggttc tgctgaagat tttgtcatga tggacatggg gcatgaatat 600
gctcttatag atggtgacga taccttatca ccgacagaga aaatcaatag aaaaatagct 660
atttccgaag aagatacgag gagaaaagct cgagctc 697

<210> 31
<211> 232
<212> PRT
<213> Chlamydia psittaci

<400> 31
Gly Phe Asp Tyr Leu Arg Asp Asn Ser Ile Ala Thr Ser Val Asp Glu
1 5 10 15

Gln Val Gly Arg Gly Phe Tyr Phe Ala Ile Ile Asp Glu Val Asp Ser
20 25 30

Ile Leu Ile Asp Glu Ala Arg Thr Pro Leu Ile Ile Ser Gly Pro Gly
35 40 45

Glu Lys His Asn Pro Val Tyr Phe Glu Leu Lys Asp Lys Val Ala Asp
50 55 60

Leu Val Gln Leu Gln Arg Glu Leu Cys Asn Gln Leu Ala Leu Glu Ala
65 70 75 80

Arg Arg Gly Leu Glu Leu Phe Leu Asp Met Asp Ile Leu Pro Lys Asp
85 90 95

Lys Lys Val Ile Glu Ala Ile Ser Glu Phe Cys Arg Ser Leu Trp Leu
100 105 110

Val Ser Lys Gly Met Pro Leu Asn Arg Val Leu Arg Arg Val Arg Glu
115 120 125

His Pro Asp Leu Arg Ala Met Ile Asp Lys Trp Asp Thr Tyr Tyr His
130 135 140

Ala Glu Gln Asn Lys Glu Glu Ser Ile Glu Lys Leu Ser Gln Leu Tyr
145 150 155 160

Ile Ile Val Asp Glu His Asn Asn Asp Phe Glu Leu Thr Asp Arg Gly
165 170 175

Met Gln Gln Trp Val Asp Lys Ala Gly Gly Ser Ala Glu Asp Phe Val
180 185 190

Met Met Asp Met Gly His Glu Tyr Ala Leu Ile Asp Gly Asp Asp Thr
195 200 205

Leu Ser Pro Thr Glu Lys Ile Asn Arg Lys Ile Ala Ile Ser Glu Glu
210 215 220

Asp Thr Arg Arg Lys Ala Arg Ala
225 230

<210> 32
<211> 2910
<212> DNA
<213> Chlamydia psittaci

<400> 32
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caaaaacttg tggataaggt caacctctat gatgagatgc tagctccttt gtctgatgag 120
gagttacgta ataaaacagc agagttaaaa aagcgttatc aggacggcga atccttagat 180

gatatgcttc ccgaggctta tgccgtagtg aaaaatgtat gcaggcgttt aacaggaact 240
cctgtagaag tgtcgggtta tcatcaaaat tgggacatgg ttccctatga tgtgcaggtt 300
ctcggtgcta tagctatgca taagggcttt ataaccgaga tgcagacagg agaggggaaa 360
actctcaccg ctgttatgcc tctatattta aatgcattga caggcaagcc tgtgcattta 420
gtcacagtga atgattatct cgctcaaagg gattgtgagt gggtcggctc tatattgcgt 480
tggttaggtt taactaccgg agtattgata tcaggatcgc ctttagaaaa aagaaaagac 540
atttatcgtt gtgacgttgt ctacggtaca gcatcagagt tcgggtttga ttatctcaga 600
gataattcta ttgcaacttc tgtggatgag caggtgggac gtgggtttta ttttgctatt 660
atcgatgaag tcgactcgat tttaattgat gaagccagaa ctcctttaat tatttctggt 720
cctggggaaa aacataatcc tgtgtatttc gaactcaaag ataaagtggc tgacctcgtt 780
cagttacaaa gggagttatg taaccagtta gctcttgaag ctagacgggg actagaattg 840
ttcttggata tggatattct tcctaaggat aaaaaagtta tcgaagctat ctccgaattt 900
tgccgtagct tatggttagt tagtaaggga atgcctttaa atcgtgtttt gcgtagagtg 960
cgcgaacacc cagatttgcg agccatgata gataaatggg atacttatta tcatgctgag 1020
caaaataaag aagagagtat agagaagcta tctcagctgt atatcattgt tgatgaacat 1080
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gataccttat caccgacaga gaaaatcaat agaaaaatag ctatttccga agaagatacg 1260
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cgcccgcaac caggtcgtcg tttttccgaa ggactgcatc aagccataga agcaaaagaa 1440
catgtcacta tccgtaagga atcacaaacg tttgctacag ttaccttaca gaatttcttc 1500
cgtctgtatg aaaaactcgc aggtatgacg ggaacagcaa ttacggaatc taaagagttt 1560
aaagagattt ataatcttta tgtattgcag gtgcccacgt ttaaagaatg tttgcgtgta 1620
gatcacaatg acgaatttta tatgacagag cgtgaaaagt accacgcgat tgttaaggaa 1680
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aaaaatcatg ctcaagaagc agagatcatt gcagcagcag gaaagctggg agctgtgact 1860
gtagctacca atatggctgg ccgtggtaca gatattaagc tggatgaaga agctgtagtt 1920
gttggaggtc tccatgttat tggtacgagt cggcaccaat cacgccgtat agataggcag 1980
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gaagatcgcc tgatgcgctt atttgcatcg cccaagttaa atgccttgat tcgtcatttc 2100
cgtcctcctg aaggagaggc tatgtcggat cctatgttca ataagctcat tgaaacagca 2160
cagaaacggg tggaagcaag aaactatact attcgaaagc atactcttga gtatgacgat 2220
gttatgaata ggcaaaggca gacgatctat gcttttcgta atgacgttat ccgctctgaa 2280
gatatctttg gtttagctaa ggaagcaata tctcatgttg cattaatgat cgcttcgttg 2340
atagtgagcc gtgatcatcc tacagggaat tctcttccta ggctggaaga atggatgaac 2400
tattctttcc cactgcaatt gaatattgaa gaattgaaaa gattgaagtc tatagatgcc 2460
attgccgaac gggttgctga tgatctcata gaagttttcc agaataagtt tgcttctatg 2520
gtgcaggaaa ttaccgaagc agccggagaa aaagtcgatg ctaatggtgt ctgtaaagat 2580
gttattcgct cggtcatgat tatgcatatc gatgagcagt ggaaaattca tcttgtagat 2640
atggatttat tacgtagtga agtaggttta cgtactgtcg gtcagaaaga ccctcttatc 2700
gaatttaaac atgagtcgtt cttactattc gaaagtctta ttcgcgatat tcgtattgct 2760
attgtaaagc atttgttccg tttagagttg acgatgacta gagaacagcg gcctcaaaat 2820
gtcgtgcctg ttgttgccac atctttccaa aataatgaaa atttcggtcc tttggaactc 2880
acagttatca gtgattctga cgatgaataa 2910

<210> 33
<211> 969
<212> PRT
<213> Chlamydia psittaci

<400> 33
Met Leu Asp Phe Leu Lys Arg Phe Phe Gly Ser Ser Gln Glu Arg Thr
1 5 10 15

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Leu Lys Lys Phe Gln Lys Leu Val Asp Lys Val Asn Leu Tyr Asp Glu
            20                  25                  30

Met Leu Ala Pro Leu Ser Asp Glu Glu Leu Arg Asn Lys Thr Ala Glu
        35                  40                  45

Leu Lys Lys Arg Tyr Gln Asp Gly Glu Ser Leu Asp Asp Met Leu Pro
    50                  55                  60

Glu Ala Tyr Ala Val Val Lys Asn Val Cys Arg Arg Leu Thr Gly Thr
65                  70                  75                  80

Pro Val Glu Val Ser Gly Tyr His Gln Asn Trp Asp Met Val Pro Tyr
                85                  90                  95

Asp Val Gln Val Leu Gly Ala Ile Ala Met His Lys Gly Phe Ile Thr
            100                 105                 110

Glu Met Gln Thr Gly Glu Gly Lys Thr Leu Thr Ala Val Met Pro Leu
        115                 120                 125

Tyr Leu Asn Ala Leu Thr Gly Lys Pro Val His Leu Val Thr Val Asn
    130                 135                 140

Asp Tyr Leu Ala Gln Arg Asp Cys Glu Trp Val Gly Ser Ile Leu Arg
145                 150                 155                 160

Trp Leu Gly Leu Thr Thr Gly Val Leu Ile Ser Gly Ser Pro Leu Glu
                165                 170                 175

Lys Arg Lys Asp Ile Tyr Arg Cys Asp Val Val Tyr Gly Thr Ala Ser
            180                 185                 190

Glu Phe Gly Phe Asp Tyr Leu Arg Asp Asn Ser Ile Ala Thr Ser Val
        195                 200                 205

Asp Glu Gln Val Gly Arg Gly Phe Tyr Phe Ala Ile Ile Asp Glu Val
    210                 215                 220

Asp Ser Ile Leu Ile Asp Glu Ala Arg Thr Pro Leu Ile Ile Ser Gly
225                 230                 235                 240

Pro Gly Glu Lys His Asn Pro Val Tyr Phe Glu Leu Lys Asp Lys Val
                245                 250                 255

Ala Asp Leu Val Gln Leu Gln Arg Glu Leu Cys Asn Gln Leu Ala Leu
            260                 265                 270

Glu Ala Arg Arg Gly Leu Glu Leu Phe Leu Asp Met Asp Ile Leu Pro
        275                 280                 285

Lys Asp Lys Lys Val Ile Glu Ala Ile Ser Glu Phe Cys Arg Ser Leu
    290                 295                 300

Trp Leu Val Ser Lys Gly Met Pro Leu Asn Arg Val Leu Arg Arg Val
305                 310                 315                 320
```

Arg Glu His Pro Asp Leu Arg Ala Met Ile Asp Lys Trp Asp Thr Tyr
325 330 335

Tyr His Ala Glu Gln Asn Lys Glu Glu Ser Ile Glu Lys Leu Ser Gln
340 345 350

Leu Tyr Ile Ile Val Asp Glu His Asn Asn Asp Phe Glu Leu Thr Asp
355 360 365

Arg Gly Met Gln Gln Trp Val Asp Lys Ala Gly Gly Ser Ala Glu Asp
370 375 380

Phe Val Met Met Asp Met Gly His Glu Tyr Ala Leu Ile Asp Gly Asp
385 390 395 400

Asp Thr Leu Ser Pro Thr Glu Lys Ile Asn Arg Lys Ile Ala Ile Ser
405 410 415

Glu Glu Asp Thr Arg Arg Lys Ala Arg Ala His Gly Leu Arg Gln Leu
420 425 430

Leu Arg Ala His Leu Leu Met Glu Arg Asp Val Asp Tyr Ile Val Arg
435 440 445

Asn Asp Gln Ile Val Ile Ile Asp Glu His Thr Gly Arg Pro Gln Pro
450 455 460

Gly Arg Arg Phe Ser Glu Gly Leu His Gln Ala Ile Glu Ala Lys Glu
465 470 475 480

His Val Thr Ile Arg Lys Glu Ser Gln Thr Phe Ala Thr Val Thr Leu
485 490 495

Gln Asn Phe Phe Arg Leu Tyr Glu Lys Leu Ala Gly Met Thr Gly Thr
500 505 510

Ala Ile Thr Glu Ser Lys Glu Phe Lys Glu Ile Tyr Asn Leu Tyr Val
515 520 525

Leu Gln Val Pro Thr Phe Lys Glu Cys Leu Arg Val Asp His Asn Asp
530 535 540

Glu Phe Tyr Met Thr Glu Arg Glu Lys Tyr His Ala Ile Val Lys Glu
545 550 555 560

Ile Ala Arg Ile His Ala Val Gly Asn Pro Ile Leu Ile Gly Thr Glu
565 570 575

Ser Val Glu Val Ser Glu Lys Leu Ser Arg Ile Leu Arg Gln Asn Arg
580 585 590

Ile Glu His Thr Val Leu Asn Ala Lys Asn His Ala Gln Glu Ala Glu
595 600 605

Ile Ile Ala Ala Ala Gly Lys Leu Gly Ala Val Thr Val Ala Thr Asn
610 615 620

Met Ala Gly Arg Gly Thr Asp Ile Lys Leu Asp Glu Glu Ala Val Val
625 630 635 640

Val Gly Gly Leu His Val Ile Gly Thr Ser Arg His Gln Ser Arg Arg
645 650 655

Ile Asp Arg Gln Leu Arg Gly Arg Cys Ala Arg Leu Gly Asp Pro Gly
660 665 670

Ser Ala Lys Phe Phe Leu Ser Phe Glu Asp Arg Leu Met Arg Leu Phe
675 680 685

Ala Ser Pro Lys Leu Asn Ala Leu Ile Arg His Phe Arg Pro Pro Glu
690 695 700

Gly Glu Ala Met Ser Asp Pro Met Phe Asn Lys Leu Ile Glu Thr Ala
705 710 715 720

Gln Lys Arg Val Glu Ala Arg Asn Tyr Thr Ile Arg Lys His Thr Leu
725 730 735

Glu Tyr Asp Asp Val Met Asn Arg Gln Arg Gln Thr Ile Tyr Ala Phe
740 745 750

Arg Asn Asp Val Ile Arg Ser Glu Asp Ile Phe Gly Leu Ala Lys Glu
755 760 765

Ala Ile Ser His Val Ala Leu Met Ile Ala Ser Leu Ile Val Ser Arg
770 775 780

Asp His Pro Thr Gly Asn Ser Leu Pro Arg Leu Glu Glu Trp Met Asn
785 790 795 800

Tyr Ser Phe Pro Leu Gln Leu Asn Ile Glu Glu Leu Lys Arg Leu Lys
805 810 815

Ser Ile Asp Ala Ile Ala Glu Arg Val Ala Asp Asp Leu Ile Glu Val
820 825 830

Phe Gln Asn Lys Phe Ala Ser Met Val Gln Glu Ile Thr Glu Ala Ala
835 840 845

Gly Glu Lys Val Asp Ala Asn Gly Val Cys Lys Asp Val Ile Arg Ser
850 855 860

Val Met Ile Met His Ile Asp Glu Gln Trp Lys Ile His Leu Val Asp
865 870 875 880

Met Asp Leu Leu Arg Ser Glu Val Gly Leu Arg Thr Val Gly Gln Lys
885 890 895

Asp Pro Leu Ile Glu Phe Lys His Glu Ser Phe Leu Leu Phe Glu Ser
900 905 910

Leu Ile Arg Asp Ile Arg Ile Ala Ile Val Lys His Leu Phe Arg Leu
915 920 925

Glu Leu Thr Met Thr Arg Glu Gln Arg Pro Gln Asn Val Val Pro Val
930 935 940

Val Ala Thr Ser Phe Gln Asn Asn Glu Asn Phe Gly Pro Leu Glu Leu
945 950 955 960

Thr Val Ile Ser Asp Ser Asp Asp Glu
965

<210> 34
<211> 577
<212> DNA
<213> Chlamydia psittaci

<400> 34
gttgatgctg cagttattcc agggaacttc gccattgcag ggggaatctg tccgtataaa 60
aacagtctat acctagaaga tgtccgtact tcccaataca ccaatgtcgt tgtcatacgt 120
gctgaagata tggaagactc gagaatgcat aaactaaaac agctattgca aagcagttct 180
gtgcaggatt tctttaatac gaaatataag gggatctttt tatcgcagta acacatctgg 240
atggcttagg gaagagttga gccaccccgt tctccgtagg tttaaggcat attgggaaac 300
gattttcttg aatttttga aaaactttga ctgtttttct tttgattatt cgaagcagat 360
gtatgtcgag tatggcggtt ttagggccca gaggtccttt cagttctcct tttacatgtt 420
ctctataccc aacccaccta aaaatgcact tgctaggttc cattcctata gttggcatat 480
acattggagc gaagcggata gccgccgttg ctcaatatca tagaatgtgt agagcgaata 540
caggagtgtc tcaggtgatt attcaggatt caggatt 577

<210> 35
<211> 76
<212> PRT
<213> Chlamydia psittaci

<400> 35
Val Asp Ala Ala Val Ile Pro Gly Asn Phe Ala Ile Ala Gly Gly Ile
1 5 10 15

Cys Pro Tyr Lys Asn Ser Leu Tyr Leu Glu Asp Val Arg Thr Ser Gln
20 25 30

Tyr Thr Asn Val Val Val Ile Arg Ala Glu Asp Met Glu Asp Ser Arg
35 40 45

Met His Lys Leu Lys Gln Leu Leu Gln Ser Ser Ser Val Gln Asp Phe
50 55 60

Phe Asn Thr Lys Tyr Lys Gly Ile Phe Leu Ser Gln
65 70 75

<210> 36
<211> 804
<212> DNA
<213> Chlamydia psittaci

<400> 36
atgaaaaaaa tcacaatact ctcgttactt gctttagcca tctctttaac aggttgttgc 60

aagaattcag aaggagtctt gcggattgcg gcgagtccca cgccacatgc agagcttctt 120
tatagtttag aaaaggaggc tcaatccctt ggattgcaat tgaaaatact tcccatagat 180
gattaccgtg tacctaaccg tttgctttta gataagcaaa tagaggcaaa ttatttccaa 240
catgaagatt tcttaaaaga tgagtgtgct cggtaccaat gcgaaggaaa acttgcgatt 300
ttggctaagg tacatttaga acctatgggt ttatattcta ataaaaccca gtctctcgaa 360
gagcttaaag tcaaggaaca gctacgtata gcggttccta tagatagaac aaacgaacaa 420
cgtgcgctag acttattgcg agactgcaat ttgattagtt acaaagaagc ttctcatcta 480
gatatcaccg caaaagatgt ctttggttgt ggagggaaaa aggtaacgat tatagagatg 540
gcagcacctt tattagtatc ttctttacca gacgttgatg ctgcagttat tccagggaac 600
ttcgccattg caggggggaat ctgtccgtat aaaaacagtc tatacctaga agatgtccgt 660
acttcccaat acaccaatgt cgttgtcata cgtgctgaag atatggaaga ctcgagaatg 720
cataaactaa aacagctatt gcaaagcagt tctgtgcagg atttctttaa tacgaaatat 780
aaggggatct ttttatcgca gtaa 804

<210> 37
<211> 267
<212> PRT
<213> Chlamydia psittaci

<400> 37
Met Lys Lys Ile Thr Ile Leu Ser Leu Leu Ala Leu Ala Ile Ser Leu
1 5 10 15

Thr Gly Cys Cys Lys Asn Ser Glu Gly Val Leu Arg Ile Ala Ala Ser
20 25 30

Pro Thr Pro His Ala Glu Leu Leu Tyr Ser Leu Glu Lys Glu Ala Gln
35 40 45

Ser Leu Gly Leu Gln Leu Lys Ile Leu Pro Ile Asp Asp Tyr Arg Val
50 55 60

Pro Asn Arg Leu Leu Leu Asp Lys Gln Ile Glu Ala Asn Tyr Phe Gln
65 70 75 80

His Glu Asp Phe Leu Lys Asp Glu Cys Ala Arg Tyr Gln Cys Glu Gly
85 90 95

Lys Leu Ala Ile Leu Ala Lys Val His Leu Glu Pro Met Gly Leu Tyr
100 105 110

Ser Asn Lys Thr Gln Ser Leu Glu Glu Leu Lys Val Lys Glu Gln Leu
115 120 125

Arg Ile Ala Val Pro Ile Asp Arg Thr Asn Glu Gln Arg Ala Leu Asp
130 135 140

Leu Leu Arg Asp Cys Asn Leu Ile Ser Tyr Lys Glu Ala Ser His Leu
145 150 155 160

Asp Ile Thr Ala Lys Asp Val Phe Gly Cys Gly Gly Lys Lys Val Thr
165 170 175

Ile Ile Glu Met Ala Ala Pro Leu Leu Val Ser Ser Leu Pro Asp Val
180 185 190

```
Asp Ala Ala Val Ile Pro Gly Asn Phe Ala Ile Ala Gly Gly Ile Cys
        195                 200                 205

Pro Tyr Lys Asn Ser Leu Tyr Leu Glu Asp Val Arg Thr Ser Gln Tyr
    210                 215                 220

Thr Asn Val Val Val Ile Arg Ala Glu Asp Met Glu Asp Ser Arg Met
225                 230                 235                 240

His Lys Leu Lys Gln Leu Leu Gln Ser Ser Ser Val Gln Asp Phe Phe
                245                 250                 255

Asn Thr Lys Tyr Lys Gly Ile Phe Leu Ser Gln
            260                 265
```

<210> 38
<211> 402
<212> DNA
<213> Chlamydia psittaci

<400> 38
catgtatttt acgcaaaaaa taaacggtat aactcctgct tacaagccgc gctataccac 60
aataatatcc cgacaaccgt gtacacaaac cttattgata tcgtgaagaa aaattcttca 120
ctaatcacga agtacttttc catcaaacaa cgatgcttaa atctaaaaga tttccatttt 180
tatgatgttt atgctcccct aagtcagtcc aaagagaaaa aatatacgtt ccaagaagct 240
gtggatctta tctatactag cctttctcct ctaggaacgg aatacattga taccttaaaa 300
caggggttaa caactcaagg ctgggtagat aaatacgaaa atcttaataa acgctccgga 360
gcctattctt cgggatgtta cgatagccac ccttatgtcc tc 402

<210> 39
<211> 134
<212> PRT
<213> Chlamydia psittaci

<400> 39
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His Val Phe Tyr Ala Lys Asn Lys Arg Tyr Asn Ser Cys Leu Gln Ala
  1               5                  10                  15

Ala Leu Tyr His Asn Asn Ile Pro Thr Thr Val Tyr Thr Asn Leu Ile
            20                  25                  30

Asp Ile Val Lys Lys Asn Ser Ser Leu Ile Thr Lys Tyr Phe Ser Ile
        35                  40                  45

Lys Gln Arg Cys Leu Asn Leu Lys Asp Phe His Phe Tyr Asp Val Tyr
    50                  55                  60

Ala Pro Leu Ser Gln Ser Lys Glu Lys Lys Tyr Thr Phe Gln Glu Ala
65                  70                  75                  80

Val Asp Leu Ile Tyr Thr Ser Leu Ser Pro Leu Gly Thr Glu Tyr Ile
                85                  90                  95

Asp Thr Leu Lys Gln Gly Leu Thr Thr Gln Gly Trp Val Asp Lys Tyr
            100                 105                 110
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Glu Asn Leu Asn Lys Arg Ser Gly Ala Tyr Ser Ser Gly Cys Tyr Asp
115 120 125

Ser His Pro Tyr Val Leu
130

<210> 40
<211> 1827
<212> DNA
<213> Chlamydia psittaci

<400> 40
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tgttgggata tcacccccct atatctaaat agaaaagcat ggaaagcaga tcttgattct 120
ttcggattaa aaacagacgg ctcacctacg tggcccgctc ttcaagcaac gcaataccaa 180
ctggacaact cagaatctct actatcctta ttaactactc tcttctctat tgagagaaaa 240
ttaaacaaac tctacgttta cgctcatctg actcatgatc aggatattac aaatcaagaa 300
ggcatcgcag atcttaaatc tatcacgcat ctacatacct tatttgccga agaaacctct 360
tgggtacaac ccgctttaac cagcctatcg gaatctctca ttgctcagca cctatcagct 420
ccctgtttag ctccttatag attctattta gagaaaatct ttagactatc tatacacaca 480
ggcactcctg gagaagaaaa aattctcgct tccgccttta ctcctcttga agtagccagt 540
aaggcatttt cttctttaag tgactctgaa attccctttg ggcaagctac agactcagaa 600
ggaaactctc acccgctttc tcatgcactg gcttcattgt atatgcaatc cacagatcgg 660
gaattacgaa aaacatccta cctagcacaa tgtgaaagat atcatagtta ccgacatacc 720
tttgctaact tactcaatgg gaaaatccaa gcccatgtat tttacgcaaa aaataaacgg 780
tataactcct gcttacaagc cgcgctatac cacaataata tcccgacaac cgtgtacaca 840
aaccttattg atatcgtgaa gaaaaattct tcactaatca cgaagtactt ttccatcaaa 900
caacgatgct taaatctaaa agatttccat ttttatgatg tttatgctcc cctaagtcag 960
tccaaagaga aaaaatatac gttccaagaa gctgtggatc ttatctatac tagcctttct 1020
cctctaggaa cggaatacat tgatacctta aaacaggggt taacaactca aggctgggta 1080
gataaatacg aaaatcttaa taaacgctcc ggagcctatt cttcgggatg ttacgatagc 1140
cacccttatg tcctcctaaa ctatacaggc accctgtatg atgtatccgt cattgcccac 1200
gaaggcggac acagtatgca ctcgtatttt agtaggaagc atcaaccttt ccatgacgct 1260
caatatccta ttttccttgc tgaaattgct tctaccttaa atgaaatgct tcttatggat 1320
tccatgctga aggagagcga ctcaaaagaa gagaaaatca ccattctgac acgatgtttg 1380
gataccatct tctctacact attccgtcag gtattattcg cctcttttga atacgatatt 1440
catcacgcag cagaacatgg ggttcctcta actgaagaat acctatcctc aacttacaag 1500
aatttacaaa atgagtttta cggagaaatt atcacatttg atgtcctgtc gaacatagaa 1560
tgggcaagaa ttcctcattt ctattacaat ttctacgtat accaatatgc aacgggcatt 1620
atagccgccc tgtgcttttt agaaaaaatt cttaacaacg aagataacgc tcttaactcc 1680
tatctcaact ttttaaaaag tggtggatca gatttcccct tagaaatctt aaaaaaatca 1740
ggattagata tgggcacagt tgagccaatc caaaaagctt tttgctttat cgagaaaaaa 1800
atccaggagc tatcatcttt aatttga 1827

<210> 41
<211> 608
<212> PRT
<213> Chlamydia psittaci

<400> 41
Met Ser Val Glu Phe Asn Lys Gln Gln Val Arg Pro Arg Ser Glu Ile
1 5 10 15

Ser Pro Gln Asp Cys Trp Asp Ile Thr Pro Leu Tyr Leu Asn Arg Lys

```
            20                  25                  30

Ala Trp Lys Ala Asp Leu Asp Ser Phe Gly Leu Lys Thr Asp Gly Ser
        35                  40                  45

Pro Thr Trp Pro Ala Leu Gln Ala Thr Gln Tyr Gln Leu Asp Asn Ser
    50                  55                  60

Glu Ser Leu Leu Ser Leu Leu Thr Thr Leu Phe Ser Ile Glu Arg Lys
65                  70                  75                  80

Leu Asn Lys Leu Tyr Val Tyr Ala His Leu Thr His Asp Gln Asp Ile
                85                  90                  95

Thr Asn Gln Glu Gly Ile Ala Asp Leu Lys Ser Ile Thr His Leu His
            100                 105                 110

Thr Leu Phe Ala Glu Glu Thr Ser Trp Val Gln Pro Ala Leu Thr Ser
        115                 120                 125

Leu Ser Glu Ser Leu Ile Ala Gln His Leu Ser Ala Pro Cys Leu Ala
    130                 135                 140

Pro Tyr Arg Phe Tyr Leu Glu Lys Ile Phe Arg Leu Ser Ile His Thr
145                 150                 155                 160

Gly Thr Pro Gly Glu Glu Lys Ile Leu Ala Ser Ala Phe Thr Pro Leu
                165                 170                 175

Glu Val Ala Ser Lys Ala Phe Ser Ser Leu Ser Asp Ser Glu Ile Pro
            180                 185                 190

Phe Gly Gln Ala Thr Asp Ser Glu Gly Asn Ser His Pro Leu Ser His
        195                 200                 205

Ala Leu Ala Ser Leu Tyr Met Gln Ser Thr Asp Arg Glu Leu Arg Lys
    210                 215                 220

Thr Ser Tyr Leu Ala Gln Cys Glu Arg Tyr His Ser Tyr Arg His Thr
225                 230                 235                 240

Phe Ala Asn Leu Leu Asn Gly Lys Ile Gln Ala His Val Phe Tyr Ala
                245                 250                 255

Lys Asn Lys Arg Tyr Asn Ser Cys Leu Gln Ala Ala Leu Tyr His Asn
            260                 265                 270

Asn Ile Pro Thr Thr Val Tyr Thr Asn Leu Ile Asp Ile Val Lys Lys
        275                 280                 285

Asn Ser Ser Leu Ile Thr Lys Tyr Phe Ser Ile Lys Gln Arg Cys Leu
    290                 295                 300

Asn Leu Lys Asp Phe His Phe Tyr Asp Val Tyr Ala Pro Leu Ser Gln
305                 310                 315                 320

Ser Lys Glu Lys Lys Tyr Thr Phe Gln Glu Ala Val Asp Leu Ile Tyr
```

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                325                 330                 335

Thr Ser Leu Ser Pro Leu Gly Thr Glu Tyr Ile Asp Thr Leu Lys Gln
            340                 345                 350

Gly Leu Thr Thr Gln Gly Trp Val Asp Lys Tyr Glu Asn Leu Asn Lys
        355                 360                 365

Arg Ser Gly Ala Tyr Ser Ser Gly Cys Tyr Asp Ser His Pro Tyr Val
    370                 375                 380

Leu Leu Asn Tyr Thr Gly Thr Leu Tyr Asp Val Ser Val Ile Ala His
385                 390                 395                 400

Glu Gly Gly His Ser Met His Ser Tyr Phe Ser Arg Lys His Gln Pro
                405                 410                 415

Phe His Asp Ala Gln Tyr Pro Ile Phe Leu Ala Glu Ile Ala Ser Thr
            420                 425                 430

Leu Asn Glu Met Leu Leu Met Asp Ser Met Leu Lys Glu Ser Asp Ser
        435                 440                 445

Lys Glu Glu Lys Ile Thr Ile Leu Thr Arg Cys Leu Asp Thr Ile Phe
    450                 455                 460

Ser Thr Leu Phe Arg Gln Val Leu Phe Ala Ser Phe Glu Tyr Asp Ile
465                 470                 475                 480

His His Ala Ala Glu His Gly Val Pro Leu Thr Glu Glu Tyr Leu Ser
                485                 490                 495

Ser Thr Tyr Lys Asn Leu Gln Asn Glu Phe Tyr Gly Glu Ile Ile Thr
            500                 505                 510

Phe Asp Val Leu Ser Asn Ile Glu Trp Ala Arg Ile Pro His Phe Tyr
        515                 520                 525

Tyr Asn Phe Tyr Val Tyr Gln Tyr Ala Thr Gly Ile Ile Ala Ala Leu
    530                 535                 540

Cys Phe Leu Glu Lys Ile Leu Asn Asn Glu Asp Asn Ala Leu Asn Ser
545                 550                 555                 560

Tyr Leu Asn Phe Leu Lys Ser Gly Gly Ser Asp Phe Pro Leu Glu Ile
                565                 570                 575

Leu Lys Lys Ser Gly Leu Asp Met Gly Thr Val Glu Pro Ile Gln Lys
            580                 585                 590

Ala Phe Cys Phe Ile Glu Lys Lys Ile Gln Glu Leu Ser Ser Leu Ile
        595                 600                 605
```

<210> 42
<211> 1517
<212> DNA
<213> Chlamydia psittaci

<400> 42
gcgttagatt cggaagagct gaaagagcaa ataaacaatc taaaagagcg tttatgggat 60
gcacaatcca ctctacaaca agatcaaaat aaactatcgc aagaacattt tgaagctgtc 120
agtgtgatca ttgatttaat caatggtgat ctgaatgata tagctgagca tacgcaacaa 180
aacttacaaa ccaaaaaaga agaagaacac gagtccgttg cccgtaagat ggtcaattgg 240
gtgtcttctg gagaagaagt gttaaataga gcccttctct acttctcaga taggaatgga 300
gaacgggaaa atttagcaga ctttttaaaa gtacagtatg ctgttcaaag agcaacgcaa 360
agagcagaac tttttgctag tatcgtagga actacggtaa gtagtataaa gacgataatg 420
accacacaat taggttaaca tggacgaatt gacgacagat ttcgataccc tcatgtcgca 480
attgaacgac gtacacttga ctaccgttgt cggtcgtata actgaagtcg tcggtatgtt 540
aattaaagct gtcgttccca atgtacgcgt tggggaggta tgcttagtta aacgttatgg 600
tatggagccg ctcgtgaccg aagtcgtcgg cttcacacaa aatttcgctt ttttatcgcc 660
actaggagaa cttactggag tcagcccttc ttcagaggtt attcccacag gtctgccttt 720
gtatatccgt gcaggtaacg gtcttttagg tcgtgtattg aatggtctgg gagaacctat 780
cgactccgag atcaaaggac ctttggttga tgttaacgaa acctaccctg tgtttcgcgc 840
tccaccagat ccattgcata gagaaaaatt aagaacaatt ttatccaccg gcgtgcggtg 900
tatcgacggt atgctcacag tcgccagagg ccagcgtata ggcatttttg ctggagctgg 960
ggtgggtaaa tcgtctctct tgggaatgat cgctagaaac gcggaagaag ccgatgtcaa 1020
tgtgattgct ctcatcggag agcggggccg agaggttcgt gaatttatcg agggcgatct 1080
cggagaagaa ggaatgaaac gttcggtgat cgtcgtctct acttcagatc aatcctcaca 1140
gttgcgatta aatgctgctt acgtaggcac cgctatagca gagtattttc gtgatcaggg 1200
caaaaccgta gttttgatga tggattctgt cacccgattt gcccgagccc taagagaagt 1260
cggtctagct gccggagaac cgccagctcg aggaggatac acaccttctg tattctcaac 1320
tttgcctagg ttattagaac gttccggagc ttcggataaa ggaacaatca cagcctttta 1380
cacagtactt gttgccgggg atgatatgaa tgaaccggtc gctgatgaag ttaaatcgat 1440
tcttgatggt cacgttgtct tgtctaacgc tttagctcag gcataccatt atcctgctat 1500
tgatgtctta gcatcta 1517

<210> 43
<211> 145
<212> PRT
<213> Chlamydia psittaci

<400> 43
Ala Leu Asp Ser Glu Glu Leu Lys Glu Gln Ile Asn Asn Leu Lys Glu
1 5 10 15

Arg Leu Trp Asp Ala Gln Ser Thr Leu Gln Gln Asp Gln Asn Lys Leu
20 25 30

Ser Gln Glu His Phe Glu Ala Val Ser Val Ile Ile Asp Leu Ile Asn
35 40 45

Gly Asp Leu Asn Asp Ile Ala Glu His Thr Gln Gln Asn Leu Gln Thr
50 55 60

Lys Lys Glu Glu Glu His Glu Ser Val Ala Arg Lys Met Val Asn Trp
65 70 75 80

Val Ser Ser Gly Glu Glu Val Leu Asn Arg Ala Leu Leu Tyr Phe Ser
85 90 95

```
Asp Arg Asn Gly Glu Arg Glu Asn Leu Ala Asp Phe Leu Lys Val Gln
            100                 105                 110

Tyr Ala Val Gln Arg Ala Thr Gln Arg Ala Glu Leu Phe Ala Ser Ile
        115                 120                 125

Val Gly Thr Thr Val Ser Ser Ile Lys Thr Ile Met Thr Thr Gln Leu
    130                 135                 140

Gly
145
```

<210> 44
<211> 669
<212> DNA
<213> Chlamydia psittaci

<400> 44
atggtagatc ctttgaagct tttcccaaag ctagactccg agaaagaaac agcttctata 60
cagaagcctt taggaactcc tttagccagt gagttacata aggaagttcc tgcattttct 120
ttagggacgg cagcagactc cttgaataaa aatatagagg atgtcaagcc taaccctatg 180
gcgatgatgc aagacagaaa ctctaacatt atcgatcctg aactggaaga ggcgttagat 240
tcggaagagc tgaaagagca aataaacaat ctaaaagagc gtttatggga tgcacaatcc 300
actctacaac aagatcaaaa taaactatcg caagaacatt ttgaagctgt cagtgtgatc 360
attgatttaa tcaatggtga tctgaatgat atagctgagc atacgcaaca aaacttacaa 420
accaaaaaag aagaagaaca cgagtccgtt gcccgtaaga tggtcaattg ggtgtcttct 480
ggagaagaag tgttaaatag agcccttctc tacttctcag ataggaatgg agaacgggaa 540
aatttagcag acttttttaaa agtacagtat gctgttcaaa gagcaacgca aagagcagaa 600
ctttttgcta gtatcgtagg aactacggta agtagtataa agacgataat gaccacacaa 660
ttaggttaa 669

<210> 45
<211> 222
<212> PRT
<213> Chlamydia psittaci

<400> 45
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Met Val Asp Pro Leu Lys Leu Phe Pro Lys Leu Asp Ser Glu Lys Glu
  1               5                  10                  15

Thr Ala Ser Ile Gln Lys Pro Leu Gly Thr Pro Leu Ala Ser Glu Leu
             20                  25                  30

His Lys Glu Val Pro Ala Phe Ser Leu Gly Thr Ala Ala Asp Ser Leu
         35                  40                  45

Asn Lys Asn Ile Glu Asp Val Lys Pro Asn Pro Met Ala Met Met Gln
     50                  55                  60

Asp Arg Asn Ser Asn Ile Ile Asp Pro Glu Leu Glu Glu Ala Leu Asp
 65                  70                  75                  80

Ser Glu Glu Leu Lys Glu Gln Ile Asn Asn Leu Lys Glu Arg Leu Trp
                 85                  90                  95
```

```
Asp Ala Gln Ser Thr Leu Gln Gln Asp Gln Asn Lys Leu Ser Gln Glu
            100                 105                 110

His Phe Glu Ala Val Ser Val Ile Ile Asp Leu Ile Asn Gly Asp Leu
        115                 120                 125

Asn Asp Ile Ala Glu His Thr Gln Gln Asn Leu Gln Thr Lys Lys Glu
    130                 135                 140

Glu Glu His Glu Ser Val Ala Arg Lys Met Val Asn Trp Val Ser Ser
145                 150                 155                 160

Gly Glu Glu Val Leu Asn Arg Ala Leu Leu Tyr Phe Ser Asp Arg Asn
                165                 170                 175

Gly Glu Arg Glu Asn Leu Ala Asp Phe Leu Lys Val Gln Tyr Ala Val
            180                 185                 190

Gln Arg Ala Thr Gln Arg Ala Glu Leu Phe Ala Ser Ile Val Gly Thr
        195                 200                 205

Thr Val Ser Ser Ile Lys Thr Ile Met Thr Thr Gln Leu Gly
    210                 215                 220
```

<210> 46
<211> 1329
<212> DNA
<213> Chlamydia psittaci

<400> 46

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atggacgaat tgacgacaga tttcgatacc ctcatgtcgc aattgaacga cgtacacttg 60
actaccgttg tcggtcgtat aactgaagtc gtcggtatgt taattaaagc tgtcgttccc 120
aatgtacgcg ttggggaggt atgcttagtt aaacgttatg gtatggagcc gctcgtgacc 180
gaagtcgtcg gcttcacaca aaatttcgct tttttatcgc cactaggaga acttactgga 240
gtcagccctt cttcagaggt tattcccaca ggtctgcctt tgtatatccg tgcaggtaac 300
ggtcttttag gtcgtgtatt gaatggtctg ggagaaccta tcgactccga gatcaaagga 360
cctttggttg atgttaacga aacctaccct gtgtttcgcg ctccaccaga tccattgcat 420
agagaaaaat taagaacaat tttatccacc ggcgtgcggt gtatcgacgg tatgctcaca 480
gtcgccagag gccagcgtat aggcattttt gctggagctg gggtgggtaa atcgtctctc 540
ttgggaatga tcgctagaaa cgcggaagaa gccgatgtca atgtgattgc tctcatcgga 600
gagcggggcc gagaggttcg tgaatttatc gagggcgatc tcggagaaga aggaatgaaa 660
cgttcggtga tcgtcgtctc tacttcagat caatcctcac agttgcgatt aaatgctgct 720
tacgtaggca ccgctatagc agagtatttt cgtgatcagg gcaaaaccgt agttttgatg 780
atggattctg tcacccgatt tgcccgagcc ctaagagaag tcggtctagc tgccggagaa 840
ccgccagctc gaggaggata cacaccttct gtattctcaa ctttgcctag gttattagaa 900
cgttccggag cttcggataa aggaacaatc acagcctttt acacagtact tgttgccggg 960
gatgatatga atgaaccggt cgctgatgaa gttaaatcga ttcttgatgg tcacgttgtc 1020
ttgtctaacg ctttagctca ggcataccat tatcctgcta ttgatgtctt agcatctatc 1080
agccgattgc tgacagcaat tgttcctgag gaacaacgac gcatcatagg aaaagcccga 1140
gaggtgctgg caaaatacaa agcaaacgaa atgcttatac gtattggaga atatcgccga 1200
gggtccgatc gtgaagtgga ttttgctata gatcacattg ataaattgaa cagattctta 1260
aagcaagata ttcatgaaaa aacaaattac gaggaagcct cgcaacagct tcgggctatt 1320
ttccgataa                                                       1329
```

<210> 47
<211> 442
<212> PRT
<213> Chlamydia psittaci

<400> 47

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Met Asp Glu Leu Thr Thr Asp Phe Asp Thr Leu Met Ser Gln Leu Asn
  1               5                  10                  15

Asp Val His Leu Thr Thr Val Val Gly Arg Ile Thr Glu Val Val Gly
             20                  25                  30

Met Leu Ile Lys Ala Val Val Pro Asn Val Arg Val Gly Glu Val Cys
         35                  40                  45

Leu Val Lys Arg Tyr Gly Met Glu Pro Leu Val Thr Glu Val Val Gly
     50                  55                  60

Phe Thr Gln Asn Phe Ala Phe Leu Ser Pro Leu Gly Glu Leu Thr Gly
 65                  70                  75                  80

Val Ser Pro Ser Ser Glu Val Ile Pro Thr Gly Leu Pro Leu Tyr Ile
                 85                  90                  95

Arg Ala Gly Asn Gly Leu Leu Gly Arg Val Leu Asn Gly Leu Gly Glu
            100                 105                 110

Pro Ile Asp Ser Glu Ile Lys Gly Pro Leu Val Asp Val Asn Glu Thr
        115                 120                 125

Tyr Pro Val Phe Arg Ala Pro Pro Asp Pro Leu His Arg Glu Lys Leu
    130                 135                 140

Arg Thr Ile Leu Ser Thr Gly Val Arg Cys Ile Asp Gly Met Leu Thr
145                 150                 155                 160

Val Ala Arg Gly Gln Arg Ile Gly Ile Phe Ala Gly Ala Gly Val Gly
                165                 170                 175

Lys Ser Ser Leu Leu Gly Met Ile Ala Arg Asn Ala Glu Glu Ala Asp
            180                 185                 190

Val Asn Val Ile Ala Leu Ile Gly Glu Arg Gly Arg Glu Val Arg Glu
        195                 200                 205

Phe Ile Glu Gly Asp Leu Gly Glu Glu Gly Met Lys Arg Ser Val Ile
    210                 215                 220

Val Val Ser Thr Ser Asp Gln Ser Ser Gln Leu Arg Leu Asn Ala Ala
225                 230                 235                 240

Tyr Val Gly Thr Ala Ile Ala Glu Tyr Phe Arg Asp Gln Gly Lys Thr
                245                 250                 255

Val Val Leu Met Met Asp Ser Val Thr Arg Phe Ala Arg Ala Leu Arg
            260                 265                 270
```

```
Glu Val Gly Leu Ala Ala Gly Glu Pro Pro Ala Arg Gly Gly Tyr Thr
        275                 280                 285

Pro Ser Val Phe Ser Thr Leu Pro Arg Leu Leu Glu Arg Ser Gly Ala
    290                 295                 300

Ser Asp Lys Gly Thr Ile Thr Ala Phe Tyr Thr Val Leu Val Ala Gly
305                 310                 315                 320

Asp Asp Met Asn Glu Pro Val Ala Asp Glu Val Lys Ser Ile Leu Asp
                325                 330                 335

Gly His Val Val Leu Ser Asn Ala Leu Ala Gln Ala Tyr His Tyr Pro
            340                 345                 350

Ala Ile Asp Val Leu Ala Ser Ile Ser Arg Leu Leu Thr Ala Ile Val
        355                 360                 365

Pro Glu Glu Gln Arg Arg Ile Ile Gly Lys Ala Arg Glu Val Leu Ala
    370                 375                 380

Lys Tyr Lys Ala Asn Glu Met Leu Ile Arg Ile Gly Glu Tyr Arg Arg
385                 390                 395                 400

Gly Ser Asp Arg Glu Val Asp Phe Ala Ile Asp His Ile Asp Lys Leu
                405                 410                 415

Asn Arg Phe Leu Lys Gln Asp Ile His Glu Lys Thr Asn Tyr Glu Glu
            420                 425                 430

Ala Ser Gln Gln Leu Arg Ala Ile Phe Arg
        435                 440
```

<210> 48
<211> 477
<212> DNA
<213> Chlamydia psittaci

<400> 48
cttcttgcag atgccgactc tgtcaacctt gcaactggat tcaacggctc cactagtgaa 60
actttcaatg ttaaacaaac agataatgct gacgggacaa catatattct aggcagcgcg 120
atcacctttg aacacataaa tcaattaaaa ccagcaaaca ctagctgttt tgctaataca 180
gctggagatc taacgtttac tgggaatcga cgacttctct atttcaataa tatttcatca 240
acagcgaaag gtgccgctat cagcacaact gcggatggta agacactcac aatatccggg 300
gctctacaac tgattttcta catgtcgcca agattggcca cgggaaatgg cgtcatttat 360
tctaatagct ctgtactcat cgagaacaat tctcaaggta gctcgggact gaataagtct 420
gcagggaaag gcgtctttat ttgttgtgag aaaagtacgg atgtgggagc tacatca 477

<210> 49
<211> 159
<212> PRT
<213> Chlamydia psittaci

<400> 49
Leu Leu Ala Asp Ala Asp Ser Val Asn Leu Ala Thr Gly Phe Asn Gly

```
 1               5                  10                  15

Ser Thr Ser Glu Thr Phe Asn Val Lys Gln Thr Asp Asn Ala Asp Gly
            20                  25                  30

Thr Thr Tyr Ile Leu Gly Ser Ala Ile Thr Phe Glu His Ile Asn Gln
        35                  40                  45

Leu Lys Pro Ala Asn Thr Ser Cys Phe Ala Asn Thr Ala Gly Asp Leu
    50                  55                  60

Thr Phe Thr Gly Asn Arg Arg Leu Leu Tyr Phe Asn Asn Ile Ser Ser
65                  70                  75                  80

Thr Ala Lys Gly Ala Ala Ile Ser Thr Thr Ala Asp Gly Lys Thr Leu
                85                  90                  95

Thr Ile Ser Gly Ala Leu Gln Leu Ile Phe Tyr Met Ser Pro Arg Leu
            100                 105                 110

Ala Thr Gly Asn Gly Val Ile Tyr Ser Asn Ser Ser Val Leu Ile Glu
        115                 120                 125

Asn Asn Ser Gln Gly Ser Ser Gly Leu Asn Lys Ser Ala Gly Lys Gly
    130                 135                 140

Val Phe Ile Cys Cys Glu Lys Ser Thr Asp Val Gly Ala Thr Ser
145                 150                 155
```

<210> 50
<211> 591
<212> DNA
<213> Chlamydia psittaci

<400> 50
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acctttgaac acataaatca attaaaacca gcaaacacta gctgttttgc taatacagct 60
ggagatctaa cgtttactgg gaatcgacga cttctctatt tcaataatat ttcatcaaca 120
gcgaaaggtg ccgctatcag cacaactgcg gatggtaaga cactcacaat atccggggct 180
ctacaactga ttttcttacat gtcgccaaga ttggccacgg gaaatggcgt catttattct 240
aatagctctg tactcatcga gaacaattct caaggtagct cgggactgaa taagtctgca 300
gggaaaggcg tctttatttg ttgtgagaaa agtacggatg tgggagctac atcaccgaca 360
ttaatcatac ggaataacgg agagtttctt actgtaggta atgcagctac tagctctgga 420
ggagcgattt atgcggagaa aatgatctta tcctcaggag gatatacaaa atttcaatcc 480
aatgttagct atgatcaagg tggggccatt gccattgctc ctaatggaga aattagtctc 540
tccgcggata aaggaaatat cgtctttgaa agaaacctta aaattgccaa c          591
```

<210> 51
<211> 197
<212> PRT
<213> Chlamydia psittaci

<400> 51
```
Thr Phe Glu His Ile Asn Gln Leu Lys Pro Ala Asn Thr Ser Cys Phe
 1               5                  10                  15
```

```
Ala Asn Thr Ala Gly Asp Leu Thr Phe Thr Gly Asn Arg Arg Leu Leu
            20                  25                  30

Tyr Phe Asn Asn Ile Ser Ser Thr Ala Lys Gly Ala Ala Ile Ser Thr
        35                  40                  45

Thr Ala Asp Gly Lys Thr Leu Thr Ile Ser Gly Ala Leu Gln Leu Ile
    50                  55                  60

Phe Tyr Met Ser Pro Arg Leu Ala Thr Gly Asn Gly Val Ile Tyr Ser
65                  70                  75                  80

Asn Ser Ser Val Leu Ile Glu Asn Asn Ser Gln Gly Ser Ser Gly Leu
                85                  90                  95

Asn Lys Ser Ala Gly Lys Gly Val Phe Ile Cys Cys Glu Lys Ser Thr
            100                 105                 110

Asp Val Gly Ala Thr Ser Pro Thr Leu Ile Ile Arg Asn Asn Gly Glu
        115                 120                 125

Phe Leu Thr Val Gly Asn Ala Ala Thr Ser Ser Gly Gly Ala Ile Tyr
    130                 135                 140

Ala Glu Lys Met Ile Leu Ser Ser Gly Gly Tyr Thr Lys Phe Gln Ser
145                 150                 155                 160

Asn Val Ser Tyr Asp Gln Gly Gly Ala Ile Ala Ile Ala Pro Asn Gly
                165                 170                 175

Glu Ile Ser Leu Ser Ala Asp Lys Gly Asn Ile Val Phe Glu Arg Asn
            180                 185                 190

Leu Lys Ile Ala Asn
        195
```

<210> 52
<211> 2040
<212> DNA
<213> Chlamydia psittaci

<400> 52
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ttatccactg ctaccaaact tcttgcagat gccgactctg tcaaccttgc aactggattc 120
aacggctcca ctagtgaaac tttcaatgtt aaacaaacag ataatgctga cgggacaaca 180
tatattctag gcagcgcgat caccttttgaa cacataaatc aattaaaacc agcaaacact 240
agctgttttg ctaatacagc tggagatcta acgtttactg ggaatcgacg acttctctat 300
ttcaataata tttcatcaac agcgaaaggt gccgctatca gcacaactgc ggatggtaag 360
acactcacaa tatccggggc tctacaactg attttctaca tgtcgccaag attggccacg 420
ggaaatggcg tcatttattc taatagctct gtactcatcg agaacaattc tcaaggtagc 480
tcgggactga ataagtctgc agggaaaggc gtctttattt gttgtgagaa aagtacggat 540
gtgggagcta catcaccgac attaatcata cggaataacg gagagtttct tactgtaggt 600
aatgcagcta ctagctctgg aggagcgatt tatgcggaga aaatgatctt atcctcagga 660
ggatatacaa aatttcaatc caatgttagc tatgatcaag gtggggccat tgccattgct 720
cctaatggag aaattagtct ctccgcggat aaaggaaata tcgtctttga aagaaacctt 780
aaaattgcca acaaacaaaa tactcccaat gccattcacc taggagacaa tgcgaaattt 840

cttcaattac gtgctgctaa caacaaagcc atatttttt atgacccgat tacaaccacg 900
ggatctgtgg cagatcggct aattattaat aactcgcaag gagaagcctc gacttacgat 960
ggggcgattg tattttctag tctcaactta ttcactcatt cccctgaatg taaactctct 1020
tcattttctc aaggtcttac tttagcggca ggatcattag ttttagaaga gggggtatgt 1080
gtacaagctc cgtcttttga tcaacgtgct cactcccaac tattcatgaa tcctgggacg 1140
aagttacaag ctacccagaa catctcggta aagaatctcc atctcaatct taatagaata 1200
gcagaagagc cggcgtatat caccacaaca gacgatgctt ctagtgtgga catttgcgga 1260
cctgtagtta tgcatataga tgatgagatc ttctataatc agacagtatt agcaaatgag 1320
ttgtctgtag agtgtttaaa tctcagttct ccacatctcg ataatatcac tattgatgac 1380
gttcccgcag tgcctatcat gacgttagaa tcgcatcgtg gttatcaagg tacatgggaa 1440
atctcttgga aagagcaacc taaacttacc tttgggaagg cgactatcgc gcctaataag 1500
cagatgcacc ttatttggaa accttctggt tacgttcctt tctcaggggg aactggagag 1560
tttacgacat ctttagtgcc taatagctta tggaatctct ttttagatac acgtttttct 1620
caacaagcga ttgagaaaca tgctgtatct tcaggtaacg gtatatggat ttcctccatg 1680
accaattctt ttcttcaagg ttctacgaac aacaaccacg gctttcgtca taagagttca 1740
ggatataccg caggggggaaa aatacaaaca cttcaagatg atatctttag tgtcagtttt 1800
tctcagctat ttgggagatc taaggatttt ggatctgcca catctaagga tacattccta 1860
tcgggctcta tctatgctca gcattcgaga cgcttacttc ctataatgag attccttgca 1920
ggaacatcaa catatagacc gcgactctta ctgagtattc ccaagaatct tcctatcaat 1980
tttgatgttc ttgtgagtta cagctatgac agtaaccaca tgaaagtaca aaaattctaa 2040

<210> 53
<211> 679
<212> PRT
<213> Chlamydia psittaci

<400> 53
Met Gln Gly Ile Leu Met Lys Asn Ser Ile Tyr Gly Val Leu Leu Phe
1 5 10 15

Ser Ser Phe Ala Leu Ser Thr Ala Thr Lys Leu Leu Ala Asp Ala Asp
20 25 30

Ser Val Asn Leu Ala Thr Gly Phe Asn Gly Ser Thr Ser Glu Thr Phe
35 40 45

Asn Val Lys Gln Thr Asp Asn Ala Asp Gly Thr Thr Tyr Ile Leu Gly
50 55 60

Ser Ala Ile Thr Phe Glu His Ile Asn Gln Leu Lys Pro Ala Asn Thr
65 70 75 80

Ser Cys Phe Ala Asn Thr Ala Gly Asp Leu Thr Phe Thr Gly Asn Arg
85 90 95

Arg Leu Leu Tyr Phe Asn Asn Ile Ser Ser Thr Ala Lys Gly Ala Ala
100 105 110

Ile Ser Thr Thr Ala Asp Gly Lys Thr Leu Thr Ile Ser Gly Ala Leu
115 120 125

Gln Leu Ile Phe Tyr Met Ser Pro Arg Leu Ala Thr Gly Asn Gly Val
130 135 140

Ile Tyr Ser Asn Ser Ser Val Leu Ile Glu Asn Asn Ser Gln Gly Ser
145 150 155 160

```
Ser Gly Leu Asn Lys Ser Ala Gly Lys Gly Val Phe Ile Cys Cys Glu
                165                 170                 175

Lys Ser Thr Asp Val Gly Ala Thr Ser Pro Thr Leu Ile Ile Arg Asn
            180                 185                 190

Asn Gly Glu Phe Leu Thr Val Gly Asn Ala Ala Thr Ser Ser Gly Gly
        195                 200                 205

Ala Ile Tyr Ala Glu Lys Met Ile Leu Ser Ser Gly Gly Tyr Thr Lys
    210                 215                 220

Phe Gln Ser Asn Val Ser Tyr Asp Gln Gly Gly Ala Ile Ala Ile Ala
225                 230                 235                 240

Pro Asn Gly Glu Ile Ser Leu Ser Ala Asp Lys Gly Asn Ile Val Phe
                245                 250                 255

Glu Arg Asn Leu Lys Ile Ala Asn Lys Gln Asn Thr Pro Asn Ala Ile
            260                 265                 270

His Leu Gly Asp Asn Ala Lys Phe Leu Gln Leu Arg Ala Ala Asn Asn
        275                 280                 285

Lys Ala Ile Phe Phe Tyr Asp Pro Ile Thr Thr Thr Gly Ser Val Ala
    290                 295                 300

Asp Arg Leu Ile Ile Asn Asn Ser Gln Gly Glu Ala Ser Thr Tyr Asp
305                 310                 315                 320

Gly Ala Ile Val Phe Ser Ser Leu Asn Leu Phe Thr His Ser Pro Glu
                325                 330                 335

Cys Lys Leu Ser Ser Phe Ser Gln Gly Leu Thr Leu Ala Ala Gly Ser
            340                 345                 350

Leu Val Leu Glu Glu Gly Val Cys Val Gln Ala Pro Ser Phe Asp Gln
        355                 360                 365

Arg Ala His Ser Gln Leu Phe Met Asn Pro Gly Thr Lys Leu Gln Ala
    370                 375                 380

Thr Gln Asn Ile Ser Val Lys Asn Leu His Leu Asn Leu Asn Arg Ile
385                 390                 395                 400

Ala Glu Glu Pro Ala Tyr Ile Thr Thr Thr Asp Asp Ala Ser Ser Val
                405                 410                 415

Asp Ile Cys Gly Pro Val Val Met His Ile Asp Asp Glu Ile Phe Tyr
            420                 425                 430

Asn Gln Thr Val Leu Ala Asn Glu Leu Ser Val Glu Cys Leu Asn Leu
        435                 440                 445

Ser Ser Pro His Leu Asp Asn Ile Thr Ile Asp Asp Val Pro Ala Val
    450                 455                 460
```

```
Pro Ile Met Thr Leu Glu Ser His Arg Gly Tyr Gln Gly Thr Trp Glu
465                 470                 475                 480

Ile Ser Trp Lys Glu Gln Pro Lys Leu Thr Phe Gly Lys Ala Thr Ile
                485                 490                 495

Ala Pro Asn Lys Gln Met His Leu Ile Trp Lys Pro Ser Gly Tyr Val
            500                 505                 510

Pro Phe Ser Gly Gly Thr Gly Glu Phe Thr Thr Ser Leu Val Pro Asn
        515                 520                 525

Ser Leu Trp Asn Leu Phe Leu Asp Thr Arg Phe Ser Gln Gln Ala Ile
    530                 535                 540

Glu Lys His Ala Val Ser Ser Gly Asn Gly Ile Trp Ile Ser Ser Met
545                 550                 555                 560

Thr Asn Ser Phe Leu Gln Gly Ser Thr Asn Asn Asn His Gly Phe Arg
                565                 570                 575

His Lys Ser Ser Gly Tyr Thr Ala Gly Gly Lys Ile Gln Thr Leu Gln
            580                 585                 590

Asp Asp Ile Phe Ser Val Ser Phe Ser Gln Leu Phe Gly Arg Ser Lys
        595                 600                 605

Asp Phe Gly Ser Ala Thr Ser Lys Asp Thr Phe Leu Ser Gly Ser Ile
    610                 615                 620

Tyr Ala Gln His Ser Arg Arg Leu Leu Pro Ile Met Arg Phe Leu Ala
625                 630                 635                 640

Gly Thr Ser Thr Tyr Arg Pro Arg Leu Leu Leu Ser Ile Pro Lys Asn
                645                 650                 655

Leu Pro Ile Asn Phe Asp Val Leu Val Ser Tyr Ser Tyr Asp Ser Asn
            660                 665                 670

His Met Lys Val Gln Lys Phe
        675
```

<210> 54
<211> 487
<212> DNA
<213> Chlamydia psittaci

<400> 54
acctcgagag aggattctct tagtgtggct ttctgtcagt tatttgcaaa agataaagac 60
taccttgtaa gcaagaacgc cgcaaacgtc tatgcgggtt ctgtatatta tcagcatgtg 120
agcaagtttg atgatctcac gcggttattt aatgggccta acacgtgttg ttcagggttt 180
tctaaagaga ttcctatttt cttggatgca caaattacct attgccacac ggccaacaac 240
atgacaacgt cctatacaga ctatcctgaa gtgaaaggtt cttggggtaa tgataccctg 300
ggcttaactt tgtctactag cgtacctatc ccggtattta gttcttctat ctttgatagt 360
tatgcaccgt ttgcaaaatt acaagttgtc tatgcgcacc aagatgactt taaagaacca 420

acaacagaag gccgggtctt tgaaagcagc gatcttctca acgtttctgt acctataggt 480
ataaaat 487

<210> 55
<211> 162
<212> PRT
<213> Chlamydia psittaci

<400> 55
Thr Ser Arg Glu Asp Ser Leu Ser Val Ala Phe Cys Gln Leu Phe Ala
1 5 10 15

Lys Asp Lys Asp Tyr Leu Val Ser Lys Asn Ala Ala Asn Val Tyr Ala
20 25 30

Gly Ser Val Tyr Tyr Gln His Val Ser Lys Phe Asp Asp Leu Thr Arg
35 40 45

Leu Phe Asn Gly Pro Asn Thr Cys Cys Ser Gly Phe Ser Lys Glu Ile
50 55 60

Pro Ile Phe Leu Asp Ala Gln Ile Thr Tyr Cys His Thr Ala Asn Asn
65 70 75 80

Met Thr Thr Ser Tyr Thr Asp Tyr Pro Glu Val Lys Gly Ser Trp Gly
85 90 95

Asn Asp Thr Leu Gly Leu Thr Leu Ser Thr Ser Val Pro Ile Pro Val
100 105 110

Phe Ser Ser Ser Ile Phe Asp Ser Tyr Ala Pro Phe Ala Lys Leu Gln
115 120 125

Val Val Tyr Ala His Gln Asp Asp Phe Lys Glu Pro Thr Thr Glu Gly
130 135 140

Arg Val Phe Glu Ser Ser Asp Leu Leu Asn Val Ser Val Pro Ile Gly
145 150 155 160

Ile Lys

<210> 56
<211> 2781
<212> DNA
<213> Chlamydia psittaci

<400> 56
atgaggcctt ctttatataa gattttaata tcgtcgacgc tgacgttacc aatatctttt 60
cacttctcgc aattgcatgc agaagtggct ttaactcaag aatctattct cgatgcaaat 120
ggagcattca gtccgcaatc tacaagcact gcgggaggaa cgatttacaa cgtcgagagt 180
gatatttcta ttgtagatgt aggacagaca gcggctcttg cttcctcagc ttttgttcag 240
actgcagaca acctaacttt caaagggaac aaccatagct tatccataac gaacgcgaat 300
gccggagcta atcctgcggg aattaacgtt aacactgccg ataagattct tacgctgaca 360
gatttttcta agttgagctt taaggaatgc ccatcttctc tagtgaatac tggaaaaggg 420

gctatgaaat ccggaggagc attaaactta gcgaataatg ccagtattct gtttgatcag 480
aactattccg ctgagaatgg tggagccatc tcttgcaaag ctttttctct aaccggctcg 540
agcaaagaaa tcagcttcac cactaactct actgcgaaaa aaggtggagc gattgctgct 600
acgggaatag ctcatctttc ggacaaccaa ggcacaatca gattttctgg gaacactgct 660
gtgaattctg ggggagcagt atattcagaa gcttctatga cgattgcagg taacaaccac 720
gttgctttta gcaacaatgc tgtttccggt tcatctgatg gttgcggtgg agctatccat 780
tgtagcaaaa caggttcagc accgaccctt actataagag ataacaaagt cttgattttt 840
gaggaaaata cttcttcagc aaaaggtgga gcgatttaca ccgataaact catattgact 900
tctggtgggc ctacggcatt tatcaataac aaagttaccc atgctacacc taagggtgga 960
gctattggta ttgctgccaa tggagaatgt agcttaaccg ctgaacatgg ggatattact 1020
tttgataata acctgatggc cacacaagac aatgctacaa taaaaagaaa tgccattaac 1080
attgaaggca atggtaaatt cgtcaactta cgtgcagcgt ctggaaagac gatttctttc 1140
tatgatccta tcacagttga aggtaatgct gctgatcttc tcactttgaa taaagctgag 1200
ggtgataaaa cgtataatgg aagaattatt ttttcaggag aaaagctcac tgaagaacaa 1260
gctgctgttg cggataacct aaagacaaca tttacacagc ctatcacttt agctgctggt 1320
gaacttgtgt tacgcagcgg tgtggaagta gaagcaaaaa cagtcgtgca aacagcagga 1380
tctttgattc tgatggatgc aggcacaaag ttatccgcaa aaacagaaga tgctacactg 1440
acgaatctgg ctattaatcc gaatacctta gatgggaaaa aattcgccgt agtcgatgcc 1500
gttgctgctg ggaagaatgt gactttatca ggtgctattg gcgttattga tcctacaggg 1560
aagttttatg aaaaccataa gctaaatgat acgttagctt taggaggaat tcaactttct 1620
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cgtgctccgc tagtactcaa tagcctttgg ggatccttta tagatttacg ttctattcaa 1860
gatgtcttgg aacgtagtgt tgatagtatt cttgagacac gtcgtggttt gtgggtctct 1920
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gaagtgaaag gttcttgggg taatgatacc ctgggcttaa ctttgtctac tagcgtacct 2340
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gtctatgcgc accaagatga ctttaaagaa ccaacaacag aaggccgggt ctttgaaagc 2460
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agctgtatga caggattggc gatcaatgac gtttcctggt taaccacagc tacgaatctt 2640
gctagacaag ctttcatagt tcgcgcgggt aaccatattg ccttaacctc tggtgttgag 2700
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ggcgctaagg tcgcgttcta a 2781

<210> 57
<211> 926
<212> PRT
<213> Chlamydia psittaci

<400> 57
Met Arg Pro Ser Leu Tyr Lys Ile Leu Ile Ser Ser Thr Leu Thr Leu
1 5 10 15

Pro Ile Ser Phe His Phe Ser Gln Leu His Ala Glu Val Ala Leu Thr
20 25 30

Gln Glu Ser Ile Leu Asp Ala Asn Gly Ala Phe Ser Pro Gln Ser Thr
35 40 45

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Ser Thr Ala Gly Gly Thr Ile Tyr Asn Val Glu Ser Asp Ile Ser Ile
     50                  55                  60

Val Asp Val Gly Gln Thr Ala Ala Leu Ala Ser Ser Ala Phe Val Gln
 65                  70                  75                  80

Thr Ala Asp Asn Leu Thr Phe Lys Gly Asn Asn His Ser Leu Ser Ile
                85                  90                  95

Thr Asn Ala Asn Ala Gly Ala Asn Pro Ala Gly Ile Asn Val Asn Thr
            100                 105                 110

Ala Asp Lys Ile Leu Thr Leu Thr Asp Phe Ser Lys Leu Ser Phe Lys
        115                 120                 125

Glu Cys Pro Ser Ser Leu Val Asn Thr Gly Lys Gly Ala Met Lys Ser
    130                 135                 140

Gly Gly Ala Leu Asn Leu Ala Asn Asn Ala Ser Ile Leu Phe Asp Gln
145                 150                 155                 160

Asn Tyr Ser Ala Glu Asn Gly Gly Ala Ile Ser Cys Lys Ala Phe Ser
                165                 170                 175

Leu Thr Gly Ser Ser Lys Glu Ile Ser Phe Thr Thr Asn Ser Thr Ala
            180                 185                 190

Lys Lys Gly Gly Ala Ile Ala Ala Thr Gly Ile Ala His Leu Ser Asp
        195                 200                 205

Asn Gln Gly Thr Ile Arg Phe Ser Gly Asn Thr Ala Val Asn Ser Gly
    210                 215                 220

Gly Ala Val Tyr Ser Glu Ala Ser Met Thr Ile Ala Gly Asn Asn His
225                 230                 235                 240

Val Ala Phe Ser Asn Asn Ala Val Ser Gly Ser Ser Asp Gly Cys Gly
                245                 250                 255

Gly Ala Ile His Cys Ser Lys Thr Gly Ser Ala Pro Thr Leu Thr Ile
            260                 265                 270

Arg Asp Asn Lys Val Leu Ile Phe Glu Glu Asn Thr Ser Ser Ala Lys
        275                 280                 285

Gly Gly Ala Ile Tyr Thr Asp Lys Leu Ile Leu Thr Ser Gly Gly Pro
    290                 295                 300

Thr Ala Phe Ile Asn Asn Lys Val Thr His Ala Thr Pro Lys Gly Gly
305                 310                 315                 320

Ala Ile Gly Ile Ala Ala Asn Gly Glu Cys Ser Leu Thr Ala Glu His
                325                 330                 335

Gly Asp Ile Thr Phe Asp Asn Asn Leu Met Ala Thr Gln Asp Asn Ala
            340                 345                 350
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Thr Ile Lys Arg Asn Ala Ile Asn Ile Glu Gly Asn Gly Lys Phe Val
355 360 365

Asn Leu Arg Ala Ala Ser Gly Lys Thr Ile Ser Phe Tyr Asp Pro Ile
370 375 380

Thr Val Glu Gly Asn Ala Ala Asp Leu Leu Thr Leu Asn Lys Ala Glu
385 390 395 400

Gly Asp Lys Thr Tyr Asn Gly Arg Ile Ile Phe Ser Gly Glu Lys Leu
405 410 415

Thr Glu Glu Gln Ala Ala Val Ala Asp Asn Leu Lys Thr Thr Phe Thr
420 425 430

Gln Pro Ile Thr Leu Ala Ala Gly Glu Leu Val Leu Arg Ser Gly Val
435 440 445

Glu Val Glu Ala Lys Thr Val Val Gln Thr Ala Gly Ser Leu Ile Leu
450 455 460

Met Asp Ala Gly Thr Lys Leu Ser Ala Lys Thr Glu Asp Ala Thr Leu
465 470 475 480

Thr Asn Leu Ala Ile Asn Pro Asn Thr Leu Asp Gly Lys Lys Phe Ala
485 490 495

Val Val Asp Ala Val Ala Ala Gly Lys Asn Val Thr Leu Ser Gly Ala
500 505 510

Ile Gly Val Ile Asp Pro Thr Gly Lys Phe Tyr Glu Asn His Lys Leu
515 520 525

Asn Asp Thr Leu Ala Leu Gly Gly Ile Gln Leu Ser Gly Lys Gly Ser
530 535 540

Val Thr Thr Thr Asn Val Pro Ser His Val Val Gly Val Ala Glu Thr
545 550 555 560

His Tyr Gly Tyr Gln Gly Asn Trp Ser Val Ser Trp Val Lys Asp Asn
565 570 575

Asn Ser Asp Pro Lys Thr Gln Thr Ala Ile Phe Thr Trp Asn Lys Thr
580 585 590

Gly Tyr Val Pro Asn Pro Glu Arg Arg Ala Pro Leu Val Leu Asn Ser
595 600 605

Leu Trp Gly Ser Phe Ile Asp Leu Arg Ser Ile Gln Asp Val Leu Glu
610 615 620

Arg Ser Val Asp Ser Ile Leu Glu Thr Arg Arg Gly Leu Trp Val Ser
625 630 635 640

Gly Ile Gly Asn Phe Phe His Lys Asp Arg Asn Ala Glu Asn Arg Lys
645 650 655

Phe Arg His Ile Ser Ser Gly Tyr Val Leu Gly Ala Thr Thr Asn Thr
660 665 670

Ser Arg Glu Asp Ser Leu Ser Val Ala Phe Cys Gln Leu Phe Ala Lys
675 680 685

Asp Lys Asp Tyr Leu Val Ser Lys Asn Ala Ala Asn Val Tyr Ala Gly
690 695 700

Ser Val Tyr Tyr Gln His Val Ser Lys Phe Asp Asp Leu Thr Arg Leu
705 710 715 720

Phe Asn Gly Pro Asn Thr Cys Cys Ser Gly Phe Ser Lys Glu Ile Pro
725 730 735

Ile Phe Leu Asp Ala Gln Ile Thr Tyr Cys His Thr Ala Asn Asn Met
740 745 750

Thr Thr Ser Tyr Thr Asp Tyr Pro Glu Val Lys Gly Ser Trp Gly Asn
755 760 765

Asp Thr Leu Gly Leu Thr Leu Ser Thr Ser Val Pro Ile Pro Val Phe
770 775 780

Ser Ser Ser Ile Phe Asp Ser Tyr Ala Pro Phe Ala Lys Leu Gln Val
785 790 795 800

Val Tyr Ala His Gln Asp Asp Phe Lys Glu Pro Thr Thr Glu Gly Arg
805 810 815

Val Phe Glu Ser Ser Asp Leu Leu Asn Val Ser Val Pro Ile Gly Ile
820 825 830

Lys Phe Glu Lys Leu Ser Tyr Gly Glu Arg Ser Ala Tyr Asp Leu Thr
835 840 845

Leu Met Tyr Ile Pro Asp Val Tyr Arg His Asn Pro Ser Cys Met Thr
850 855 860

Gly Leu Ala Ile Asn Asp Val Ser Trp Leu Thr Thr Ala Thr Asn Leu
865 870 875 880

Ala Arg Gln Ala Phe Ile Val Arg Ala Gly Asn His Ile Ala Leu Thr
885 890 895

Ser Gly Val Glu Met Phe Ser Gln Phe Gly Phe Glu Leu Arg Ser Ser
900 905 910

Ser Arg Asn Tyr Asn Val Asp Leu Gly Ala Lys Val Ala Phe
915 920 925

<210> 58
<211> 559
<212> DNA
<213> Chlamydia psittaci

<400> 58
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tttcatcaga ttcctggtct gaagatcata gaaattgttt ttttagccct accgtttact 180
tgtcacgcta tcctaggtat tttctatctt tttcaagcgc aaactaattc acgggcttct 240
gacggcagaa aacccgcgtt aatctatgcg agaaatcttg cctatacttg gcagagaaga 300
actgcttgga ttttactttt cggtcttatt tttcacgtag ttcagtttcg ttttcttcgt 360
tatcctattc atgtagagct gcatgggcaa acatactatg ttgtcgatat tgacgcttct 420
cggtatgcgg cgatagtgcg gggtacacaa ggatttttta ctataaattt ttcagctcct 480
caacttgaaa cgattcgttt ggataaagag gatcttgacg gcagcgcagt ttctcaatta 540
ttagacagaa aagcgtatc 559

<210> 59
<211> 186
<212> PRT
<213> Chlamydia psittaci

<400> 59
Cys Val His Ser Leu Ala Gly Val Ala Phe Thr Leu Phe Leu Cys Glu
1 5 10 15

His Met Phe Thr Asn Met Leu Ala Ser Ser Tyr Phe Lys Glu Gly Ser
20 25 30

Gly Phe Val Gln Leu Val Ser Lys Phe His Gln Ile Pro Gly Leu Lys
35 40 45

Ile Ile Glu Ile Val Phe Leu Ala Leu Pro Phe Thr Cys His Ala Ile
50 55 60

Leu Gly Ile Phe Tyr Leu Phe Gln Ala Gln Thr Asn Ser Arg Ala Ser
65 70 75 80

Asp Gly Arg Lys Pro Ala Leu Ile Tyr Ala Arg Asn Leu Ala Tyr Thr
85 90 95

Trp Gln Arg Arg Thr Ala Trp Ile Leu Leu Phe Gly Leu Ile Phe His
100 105 110

Val Val Gln Phe Arg Phe Leu Arg Tyr Pro Ile His Val Glu Leu His
115 120 125

Gly Gln Thr Tyr Tyr Val Val Asp Ile Asp Ala Ser Arg Tyr Ala Ala
130 135 140

Ile Val Arg Gly Thr Gln Gly Phe Phe Thr Ile Asn Phe Ser Ala Pro
145 150 155 160

Gln Leu Glu Thr Ile Arg Leu Asp Lys Glu Asp Leu Asp Gly Ser Ala
165 170 175

Val Ser Gln Leu Leu Asp Arg Lys Ala Tyr
180 185

<210> 60

<211> 687
<212> DNA
<213> Chlamydia psittaci

<400> 60
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agctttgttt tacgttgtgt tcattcttta gcaggagttg catttacgtt gtttctctgt 120
gagcatatgt ttaccaatat gcttgcttct tcttatttta aggaaggcag tggttttgtt 180
cagttagtga gcaaatttca tcagattcct ggtctgaaga tcatagaaat tgtttttta 240
gccctaccgt ttacttgtca cgctatccta ggtattttct atctttttca agcgcaaact 300
aattcacggg cttctgacgg cagaaaaccc gcgttaatct atgcgagaaa tcttgcctat 360
acttggcaga gaagaactgc ttggatttta cttttcggtc ttatttttca cgtagttcag 420
tttcgttttc ttcgttatcc tattcatgta gagctgcatg ggcaaacata ctatgttgtc 480
gatattgacg cttctcggta tgcggcgata gtgcggggta cacaaggatt ttttactata 540
aatttttcag ctcctcaact tgaaacgatt cgtttggata aagaggatct tgacggcagc 600
gcagtttctc aattattaga cagaaaagcg tatctcctga ctcctaatgt tggaccgctt 660
ttctttatgt tgttcgggat tctttag 687

<210> 61
<211> 228
<212> PRT
<213> Chlamydia psittaci

<400> 61
Met Met Asn Glu Lys Glu Ser Cys Ser Glu Ala Thr Gln Arg Ser Trp
1 5 10 15

Lys Tyr Tyr Thr Ser Phe Val Leu Arg Cys Val His Ser Leu Ala Gly
20 25 30

Val Ala Phe Thr Leu Phe Leu Cys Glu His Met Phe Thr Asn Met Leu
35 40 45

Ala Ser Ser Tyr Phe Lys Glu Gly Ser Gly Phe Val Gln Leu Val Ser
50 55 60

Lys Phe His Gln Ile Pro Gly Leu Lys Ile Ile Glu Ile Val Phe Leu
65 70 75 80

Ala Leu Pro Phe Thr Cys His Ala Ile Leu Gly Ile Phe Tyr Leu Phe
85 90 95

Gln Ala Gln Thr Asn Ser Arg Ala Ser Asp Gly Arg Lys Pro Ala Leu
100 105 110

Ile Tyr Ala Arg Asn Leu Ala Tyr Thr Trp Gln Arg Arg Thr Ala Trp
115 120 125

Ile Leu Leu Phe Gly Leu Ile Phe His Val Val Gln Phe Arg Phe Leu
130 135 140

Arg Tyr Pro Ile His Val Glu Leu His Gly Gln Thr Tyr Tyr Val Val
145 150 155 160

Asp Ile Asp Ala Ser Arg Tyr Ala Ala Ile Val Arg Gly Thr Gln Gly
165 170 175

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Phe Phe Thr Ile Asn Phe Ser Ala Pro Gln Leu Glu Thr Ile Arg Leu
            180                 185                 190

Asp Lys Glu Asp Leu Asp Gly Ser Ala Val Ser Gln Leu Leu Asp Arg
        195                 200                 205

Lys Ala Tyr Leu Leu Thr Pro Asn Val Gly Pro Leu Phe Phe Met Leu
    210                 215                 220

Phe Gly Ile Leu
225
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